ILRAD 1982

Annual Report of the International Laboratory for Research on Animal Diseases

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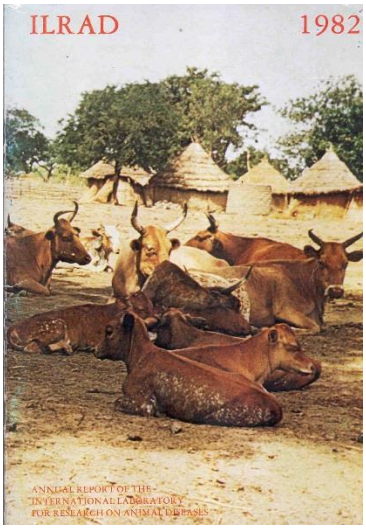

The International Laboratory for Research on Animal Diseases (ILRAD) was established in 1973 with a mandate to develop effective control measures for livestock diseases which seriously limit world food production. ILRAD's research program focuses on African animal trypanosomiasis and East Coast fever, a form of theileriosis.

ILRAD is one of 13 centres in a worldwide agricultural research network sponsored by the Consultative Group on International Agricultural Research (CGIAR). In 1982, ILRAD's research and training activities were funded by the World Bank (International Bank for Reconstruction and Development–IBRD), the United Nations Development Program (UNDP), the International Atomic Energy Agency (IAEA), and the governments of Australia, Belgium, Canada, the Federal Republic of Germany, the Netherlands, Norway, Sweden, Switzerland, the United Kingdom and the United States of America..

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Annual Report of the International Laboratory for Research on Animal Diseases

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Foreword

ILRAD—the International Laboratory for Research on Animal Diseases—was formally initiated 10 years ago, on 17 September 1973, when the Government of Kenya signed a memorandum of agreement with the Rockefeller Foundation acting on behalf of the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is an association of private foundations, national governments and regional and international organizations which support a network of agricultural research centres and programs around the world. The agreement with Kenya set out the mandate, the activities and the institutional arrangements for ILRAD's establishment.

This agreement emphasized the importance of livestock production as a source of food supply, particularly on the African continent. The authors stressed the importance of two diseases which severely limit animal production in Africa—trypanosomiasis and East Coast fever (ECF), a form of theileriosis. Efforts to improve livestock productivity by upgrading existing herds, introducing better management systems and developing grazing resources will depend very largely on progress in bringing these two diseases under control.

The animal health problems which led to ILRAD's establishment 10 years ago are, in certain areas, still as pressing as ever. Food production in Africa is actually falling, while population growth figures are the highest in the world. We can no longer expect to increase food supplies substantially simply by bringing additional land under cultivation or by dramatic improvements in farming technology: new opportunities in both these areas are limited. Yet in Africa and elsewhere in the world, millions of cattle, sheep and goats die each year from disease. And substantial productivity losses and treatment costs are incurred when animals are diseased, even if they survive.

The two most important livestock diseases on the African continent are still trypanosomiasis and ECF. Scientists from many countries have been working for decades to develop effective methods to control these diseases, but this task has proven very difficult due to the complex nature of the diseases and their vectors.

ILRAD's laboratories and support facilities were largely completed and occupied 5 years ago. The complex at Kabete, near Nairobi, was inaugurated on 5 April 1978 by President Daniel arap Moi, then Vice-President of Kenya. In 5 years of operation, ILRAD scientists have made substantial progress, both in defining the nature of the problems to be solved and in initiating the research required for their solution. However, there are still no quick or easy solutions in sight. We must therefore review our programs and plans—regularly and carefully—to ensure that we are adopting the right approaches. We must also consider what we can do now to improve the control of these diseases, with existing knowledge and the research results obtained to date, while we continue to search for effective long-term solutions.

These issues were given full attention in 1982, with the aim of making ILRAD's research programs as relevant and effective as possible. We have moved away from the former individual laboratory organization, and attention now concentrates on projects with clear disease-control objectives.

Research on theileriosis has been organized in three project areas. One area is an expanded ECF epidemiology project, based on the application of existing knowledge to improve control of the disease. Earlier work had shown that it is possible to immunize cattle against ECF by infecting them with live parasites and treating them with a tetracycline antibiotic. This method has not been fully exploited in the field because its value has been in doubt on several counts. Concern centred on the problem of strain differences and on the fact that animals immunized in this way might retain live parasites in their bloodstream and thus act as disease carriers. It has now been shown that cattle which recover naturally from ECF can also retain parasites in their bloodstream, so the occasional retention of parasites in the blood of immunized animals is no longer considered such a serious problem. ILRAD's theileriosis epidemiology project is exploring the practical value of this 'infection and treatment' method of immunization at several locations in Kenya.

While there is some progress in this area, large-scale immunization using live *Theileria* parasites will probably never be fully satisfactory because it involves a certain amount of risk and because it is difficult to obtain enough live parasites. ILRAD's other theileriosis research projects are pursuing two possible alternatives for the development of a more acceptable vaccine. These are antigens from *Theileria* sporozoites, the form of the parasite which is transmitted to cattle from infected ticks, and antigens from *Theileria* schizonts, the form which develops in the cells of infected cattle.

The trypanosomiasis research program has also been organized into three project areas. Again, one component is an epidemiology project. Techniques developed in the laboratory to identify and categorize trypanosome isolates are being applied in a field situation at the Kenya coast where the number of trypanosome strains is likely to be limited. Results from this work will allow us to assess the value of attempting to develop a vaccine based on tsetse transmitted trypanosome antigens. Such an approach might be successful in situations where there are only a few parasite strains. This work is particularly relevant to *Trypanosoma brucei gambiense* infections in man and may shed light on the possible role of domestic livestock as reservoirs for this pathogen.

Another component of ILRAD's trypanosomiasis epidemiology project involves collaboration with the International Livestock Centre for Africa (ILCA) and the International Centre of Insect Physiology and Ecology (ICIPE) in studying the productivity of trypanotolerant livestock at several sites in Central and West Africa. ILRAD's role is to help train field staff involved in the research network and to standardize techniques for assessing levels of tsetse challenge and the incidence of trypanosomiasis.

Research on trypanosomiasis in the laboratory is broadly based. We are following a number of promising research leads, but lines of work with assured practical applications are not easy to identify. One major research area centres on the development of methods to control the parasites themselves. The other area concentrates on improving the responses of infected animals.

The introduction of a project structure to the research program at ILRAD has required few changes in existing facilities or staff. We now have one immunology laboratory and two immuno-parasitology laboratories, dealing separately with theileriosis and trypanosomiasis. The

established laboratories continue their work in cell culture, molecular biology, biochemistry, pathology and electron microscopy.

Senior members of staff have been designated as project leaders to coordinate work in each area, under the guidance of the Director of Research who was appointed in December. While these changes were being introduced, we continued to make steady research progress, as will be described in the following sections of this report.

With the modern facilities and multidisciplinary staff assembled at ILRAD, there are many opportunities to train young scientists, technicians and field workers without serious diversion from research objectives. The program of individual training in the laboratories and the schedule of courses, conferences and workshops are both expanding steadily, as will be described in detail further on. By widely distributing information on training opportunities, we are ensuring that participation is available to individuals from as many countries as possible. To let more people know what we are doing and what assistance we can offer, we have prepared articles for outside publication and brochures in English and French describing various aspects of the research and training programs. The annual report is also now produced in both languages.

In times of financial stringency, there is always concern that funds are put to the most effective use, and possibilities for effective collaboration become particularly important. ILRAD scientists are involved in a wide range of collaborative projects with colleagues in Kenya, in other parts of Africa, in Europe and North America. The research institutes, government projects and university departments involved in these collaborative projects are mentioned throughout this report.

ILRAD's research program is guided by an international Board of Directors under the leadership of the Chairman, Dr K.F. Wells. Two members of the Board retired in 1982—Dr B.K. Na'isa and Prof Dr D. Zwart. Their substantial contribution to ILRAD's development over the years has been greatly appreciated. I would like to welcome three new Board members who were appointed in 1982—Dr D.M. Chavanduka, Prof E.N.W. Oppong and Prof W.R. Pritchard.

Scientists and livestock development specialists from many parts of the world visited ILRAD in the course of the year. The Kenyan Minister for Livestock Development, the Hon Mr Paul Ngei, visited ILRAD in April to open the annual meeting of the Board of Directors. In October, the Minister for Regional Development, Science and Technology, the Hon Mr Nicholas Biwott, came to ILRAD to open a Training Course on the Use of Immunological Methods in the Diagnosis of Haemoprotozoan Diseases. Other important visitors included their Excellencies the Ambassadors of Belgium, the Netherlands and Switzerland and the High Commissioners of Australia and Canada.

I would like to take this opportunity to thank the donors who have provided the financial support necessary to carry out our research and training activities. Over the years, funds have been provided by the World Bank (International Bank for Reconstruction and Development—IBRD), the United Nations Development Program (UNDP), the International Atomic Energy Agency (IAEA), the Rockefeller Foundation and the governments of Australia, Belgium, Canada, the Federal Republic of Germany, Ireland, the Netherlands, Norway, Sweden, Switzerland, the

United Kingdom and the United States of America. In addition, it is a pleasure to acknowledge the continuing friendship and support of the people and Government of Kenya.

In conclusion, after 5 years of full operation, we are beginning to see positive results from our research and training programs. ILRAD is already carrying out epidemiology projects in field situations where research findings from the laboratory are being applied in a practical way. We are training scientists, technicians and field workers from many countries who will provide a nucleus of personnel to improve the diagnosis and control of trypanosomiasis and theileriosis. We look forward to another active and productive year of research and training in 1983.

A R Gray

Director General

ILRAD

Theileriosis

Theileriosis is a complex of parasitic diseases which affects cattle, and sometimes sheep, goats and domestic buffalo, in Africa, the Middle East and Asia. Figure 1 shows the distribution of the three major *Theileria* species which affect cattle throughout the world. Research at ILRAD concentrates primarily on ECF, the form of theileriosis which causes substantial losses among cattle in East and Central Africa.

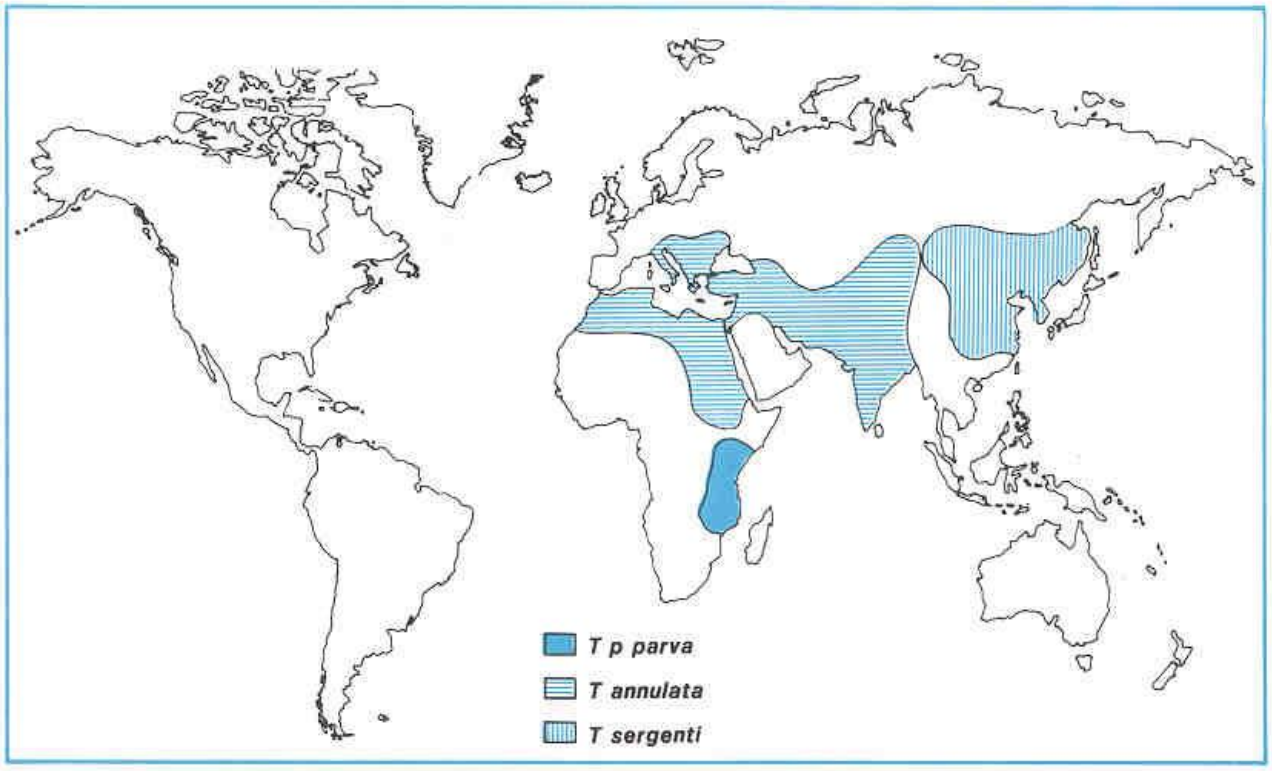

Figure 1. *World distribution of* Theileria *parasites*.

ECF is caused by the protozoan parasite *Theileria parva,* transmitted by the brown ear tick *Rhipicephalus appendiculatus.* Two subspecies of the parasite are found in Kenya, *T parva parva* which occurs in cattle and *T p lawrencei* which normally occurs in buffalo but can cause severe infections in cattle. Two related species, *T taurotragi* and *T mutans,* may also cause infections in cattle.

At present ECF is controlled primarily by dipping or spraying susceptible animals with acaricides to kill the tick vectors. In areas of heavy tick infestation, cattle must be dipped or sprayed at least twice a week. Drugs to cure ECF are being developed by commercial firms, but neither frequent treatment with acaricides nor regular drug treatment is feasible for many African livestock producers. Tick resistance to currently available acaricides is also potentially a serious problem.

The life cycle of the *T p parva* parasite is depicted in Figure 2. Ticks may feed on cattle three times during their lives—as larvae, as nymphs and as adults. ECF sporozoites develop in the salivary glands of infected ticks and are transmitted to cattle when the ticks next feed. These sporozoites invade bovine lymphoid cells where they develop into macroschizont forms which multiply rapidly. The infected lymphocytes are transformed into enlarged blast cells, which are stimulated to multiply along with the parasites. Thus, the number of parasitized cells increases rapidly. In the final stages of the disease, large number of lymph cells are destroyed, and usually the animal dies.

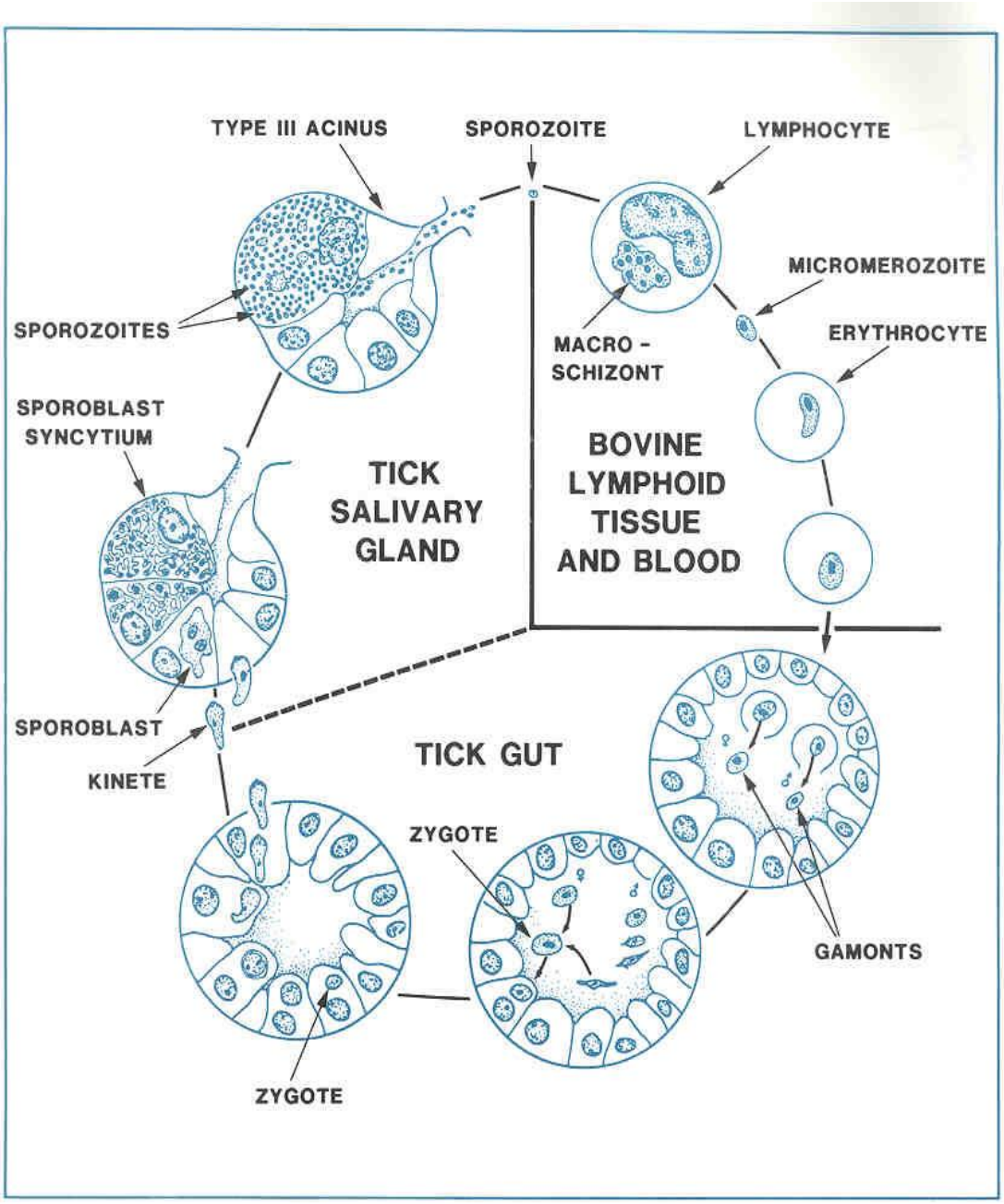

Figure 2. *Lifecycle of* T p parva. *Sporozoltes are transmitted to the bovine host in tick saliva. They enter the lymphocytes and develop into macroschizonts. After a period of nuclear division, these give rise to numerous micromerozoites which escape from the lymphocytes and enter the erythrocytes where they are ingested by feeding ticks. In the tick gut the parasites differentiate into male and female gamonts which fuse to form zygotes. These enter the cell lining of the gut where they differentiate into kinetes. The kinetes traverse the gut wall and become free in the tick's body cavity. They move to the salivary gland and enter one cell type, the E-cell of the type III acinus. Here they form an elaborate intracellular sporoblast syncytium which undergoes segmental fission and gives rise to 30,000 to 50,000 sporozoltes. These are introduced with the saliva into a new mammalian host, initiating a new cycle of parasite development.*

During the course of infection, some of the macroschizonts change to microschizonts. These give rise to micromerozoites which are released and infect red blood cells. The parasites are then transmitted back to ticks which ingest the infected cells.

Mortality rates in fully susceptible cattle can approach 100%. However, animals which recover from infection show long-lasting immunity to the same or similar disease strains. This resistance to reinfection suggests that prospects are promising for the development of an ECF vaccine. The precise mechanisms of immunity remain unclear, but it is likely that both antibody production and cell-mediated immune responses play a role.

Scientists at ILRAD are studying both the sporozoite and the macroschizont stages of parasite development and associated host responses in an effort to develop a safe and effective ECF vaccine. ILRAD's third project area, theileriosis epidemiology, includes the identification and classification of disease strains isolated in the field, the improvement of experimental immunization procedures based on infection with live parasites and antibiotic treatment, and a collaborative investigation of the role of buffalo and other wild animals as disease carriers. Collaborative research was also carried out in 1982 on immune responses to tick saliva and other tick proteins and on the possibility of immunizing cattle against the tick vector.

Towards a sporozoite vaccine

The development of sprozoites in the tick

Electron microscope studies at ILRAD have concentrated on the development of *Theileria* sporozoites in tick salivary glands. A better understanding of this process could help increase yields of sporozoites from infected ticks. Eventually, these studies could also make it possible to isolate sporozoite antigen mRNA as a basis for producing the antigen in vitro.

Work in 1981 concentrated primarily on *T p parva.* It was extended in 1982 to cover the related species and subspecies, *T taurotragi* and *T p lawrencei*, more fully. This research has shown that sporozoites do not develop by repeated binary fission, as previously assumed. Rather, in the E cell of the type III acinus, a *Theileria* sporoblast develops into a syncytium, a large, ramifying mass of parasite protoplasm. Thousands of nuclei proliferate throughout the syncytium, forming sporozoites in an episode of terminal fission. Detailed studies in 1982 showed that these nuclei

are produced by mitotic division involving dispersal and reconstitution of the nuclear envelope. The divisions are not precisely synchronized.

Attention has also focused on the development of cellular organelles in the sporozoite which may play an important role when the parasite enters and develops in a bovine lymph cell. *Theileria* sporozoites do not necessarily contact and enter bovine lymphocytes at the point where the rhoptries are attached to their surface membrane. In this they differ from other intracellular protozoan parasites. The role of the rhoptries in the initiation and development of *Theileria* infections remains unclear. Electron microscope studies in 1982 showed that the rhoptries arise near the nuclei in the *Theileria* syncytium during the final nuclear division, in close association with the spindle-pole bodies. They subsequently attach to a neighbouring subplasmalemmal dense plaque which transforms into a membrane-bounded cistern (see Figure 3).

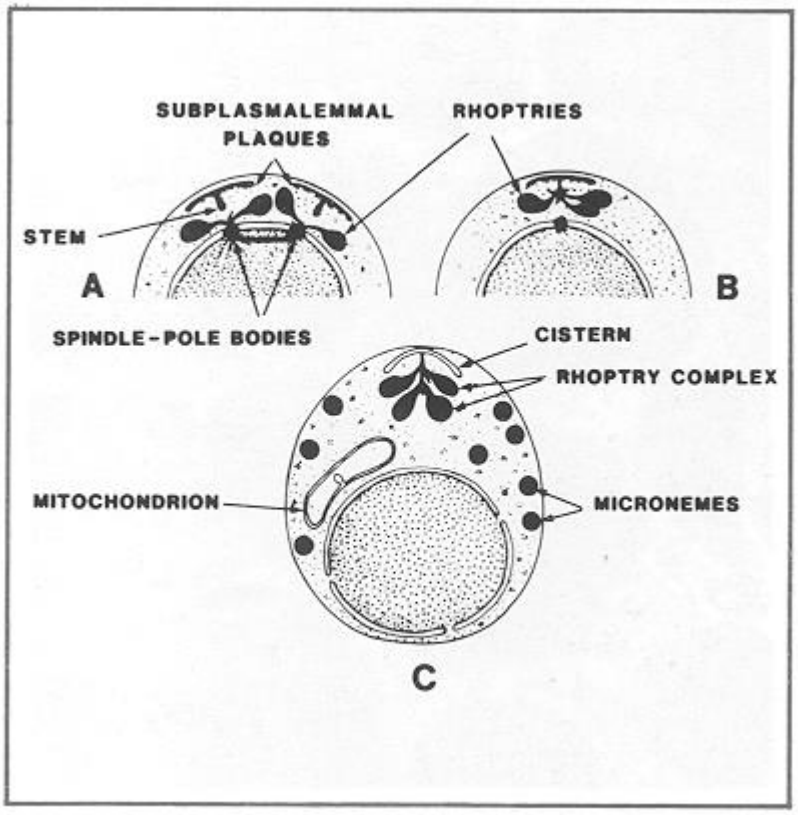

Figure 3. *Schematic depiction of the origin of the rhoptries in the development of a* Theileria *sporozoite. A: rhoptries appear to arise by polymerization of molecular subunits and are initially arranged radially around the spindle-pole bodies during the final nuclear division in the sporoblast syncytium. B: their tapering ends later attach to a stem projecting inward from a subplasmalemmal dense plaque. C: the dense plaque is subsequently transformed into a membrane-bounded cistern closely associated with the membrane of the sporozoite.*

Small secretory granules, called micronemes, are found in sporozoite cytoplasm. They may play a role in the dissolution of the host cell membrane after the entry of the parasite. Research in

1982 showed that the micronemes form after the rhoptries and increase in size and number during the maturation of the sporozoite. No evidence was found for their derivation from the rhoptries: rather, they appear to be assembled independently from molecular precursors. The possible role of the micronemes in the parasite's evasion of host cell defence mechanisms is now being studied in more detail.

Preliminary investigations in 1981 suggested that the ultrastructure of *T p parva*, *T p lawrencei* and *T taurotragi* sporozoites may be sufficiently distinctive to identify the species of parasite found in feeding ticks. Sporozoites of different species and subspecies are probably more difficult to distinguish at a later stage when they are released into the mammalian host. In 1982, sporozoites harvested from infected ticks were examined with the electron microscope, and minor but consistent differences were found between the three *Theileria* species and subspecies in terms of shape, electron density, residual bodies and number and kind of micronemes.

T taurotragi sporozoites, shown in Figure 4, are quite variable in shape and commonly possess a single tail-like process. Their surface coat is thicker and their rhoptries are larger than those of the other species. Figure 5 shows *T p lawrencei* sporozoites. They are more dense and more variable in shape than *T p parva* sporozoites and often contain multivesicular bodies not seen in the other species. They also develop micronemes at a later stage. Sporozoites of *T p parva* are uniformly ovoid or spherical, as shown in Figure 6. Their cytoplasm is less dense and their surface coat less well developed than the other two species. *Theileria* sporozoites can also be distinguished by electron microscope examination from comparable developmental stages of other parasites, such as *Babesia*.

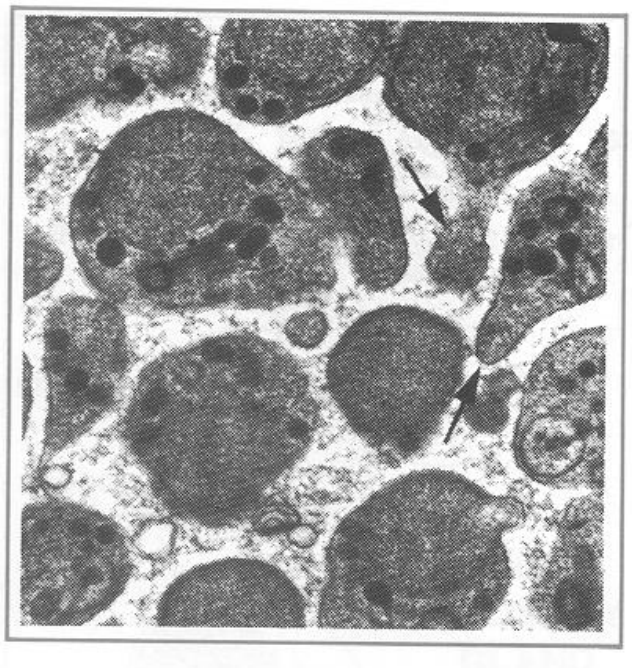

Figure 4. *Newly formed sporozoites of* T taurotragi *are highly variable in shape. Many have tail-like projections (see at arrows).*

Figure 5. *Sporozoites of* T p lawrencei *are variable in form, but less so than those of* T taurotragi. *The mitochondrion is small and micronemes appear relatively late*

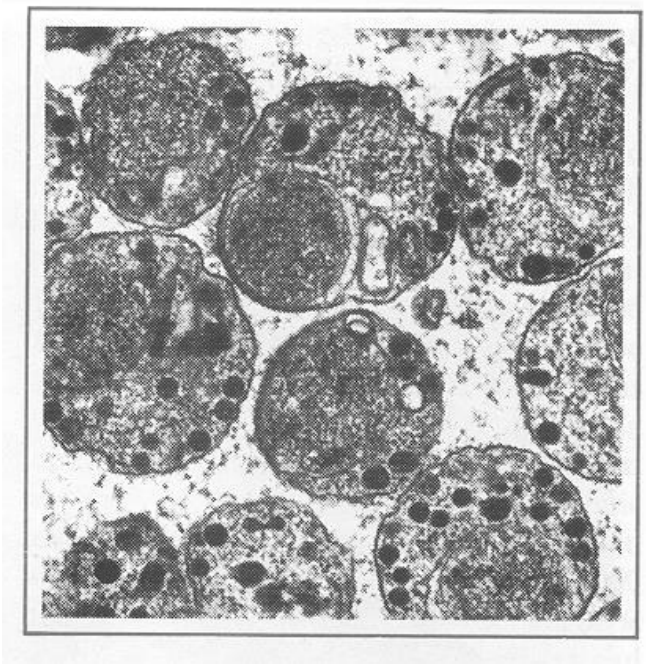

Figure 6. *Sporozoites of* T p parva *are more uniformly round or oval than sporozoites of* T taurotragi *or* T p lawrencei. *They appear slightly larger, their cytoplasm is less dense and their micronemes are more numerous than in the other two species.*

Sporozoite antigens and immune responses

Scientists at ILRAD have shown that cattle infected with *T p parva* produce neutralizing antibodies against parasite sporozoites. In 1982, anti-sporozoite antibodies were taken from 12 cattle infected with *T p parva* Muguga, a strain isolated at the former East African Veterinary Research Organization. These antibodies were tested in vitro for their ability to neutralize sporozoites of five different East African *Theileria* strains—*T p parva* Uganda, *T p lawrencei, T p parva* Kiambu 5, *T p parva* Marikebuni and *T p parva* Kabete 1. The antibodies raised against the Muguga strain neutralized the infectivity of sporozoites of all these strains. Similar observations were recorded using anti-sporozoite antibodies raised against *T p parva* Uganda and *T p lawrencei*. These results were unexpected, as some of these strains are known not to cross protect.

In a further study, 6 of the 12 original cattle were challenged repeatedly with *T p parva* Uganda and the other 6 with *T p lawrencei*. Those animals with high titres of anti-sporozoite antibodies were completely protected, while those with low levels of antibodies became infected, suggesting the role of antibody in the protective immune response. It was concluded that either a common protective antigen is found on sporozoites of all the strains covered in the study, or, if several antigens are involved, the relevant ones are represented on every strain.

The next step was to determine whether one antigen or a mixture of antigens stimulated the observed immunity and to identify precisely the antigen(s) involved. This work has been undertaken by raising monoclonal antibodies against the *Theileria* sporozoites. Monoclonal antibodies are produced experimentally by creating hybrid cells from the fusion of mouse spleen cells, which produce antibodies, and mouse myeloma cells, derived from bone-marrow. The hybrid cells are capable of growing and dividing in culture and are primed to respond to specific parasite antigens. The development of this technology has been an important component of research work at ILRAD, with many applications in the study of both theileriosis and trypanosomiasis.

To produce monoclonal antibodies to sporozoite antigens, purified sporozoite preparations were used as starting and screening material. As part of this project, ILRAD immunologists have developed a new method for purifying sporozoites. Scientists producing monoclonal antibodies in the past have most often used homogenates of whole infected ticks as starting material, partly purified by successive steps of centrifugation and sometimes by Percoll density gradients. When examined under the electron microscope, however, these. preparations prove to be highly heterogeneous, containing a variety of components, such as secretory granules, mitochondria and secretions from the ticks' Malpighian tubules, which are similar to sporozoites in terms of size and specific density. With the new technique, material from infected tick salivary glands is separated by passage through columns of diethylaminoethyl cellulose. The purity of the fractions obtained is monitored with the electron microscope.

Using sporozoites purified in this way, five highly specific IgM monoclonal antibodies were produced against *T p parva* Muguga. All five antibodies appear to recognize the same antigen. They also neutralize the infectivity of *T p parva* Uganda and *T p lawrencei* sporozoites as well as those of the Muguga strain, suggesting that sporozoites of these strains share a common antigen.

Since these three strains were isolated from widely separated locations, it is possible that their common antigen may be found in other East African *Theileria* populations. This suggests that it might be possible to develop a vaccine based on this antigen which would protect cattle over a wide area. With this aim, scientists are now testing the five antibodies against other *Theileria* strains from different locations.

The method for purifying sporozoites which was developed as part of this project has a number of other potentially useful applications. Cattle can now be immunized experimentally with much purer sporozoite preparations than were available in the past. If sporozoite production can be scaled up to yield larger numbers, it will also be possible for the first time to study the biochemistry of the sporozoite surface membrane and organelles.

Another technique was developed in 1982 for screening monoclonal antibodies which recognize sporozoite antigens. Monoclonal antibodies raised in mice are screened using an immuno-autoradiography assay system which makes it possible to distinguish precisely which antibodies recognize sporozoite antigens. Cryostat sections are prepared from infected tick salivary glands and the monoclonal antibodies are added. These bind to antigens in the preparation. A radioactive-labeled anti-mouse antibody is then added which binds to the mouse-produced monoclonal antibodies. The preparation is stained with Feulgen to reveal the location of parasite DNA; this is done to ascertain which salivary gland acini contain parasites. The preparation is then covered with a photographic emulsion which is exposed wherever the radioactive-labeled anti-mouse antibody adheres to a sporozoite antigen/monoclonal antibody complex, as shown in Figure 7.

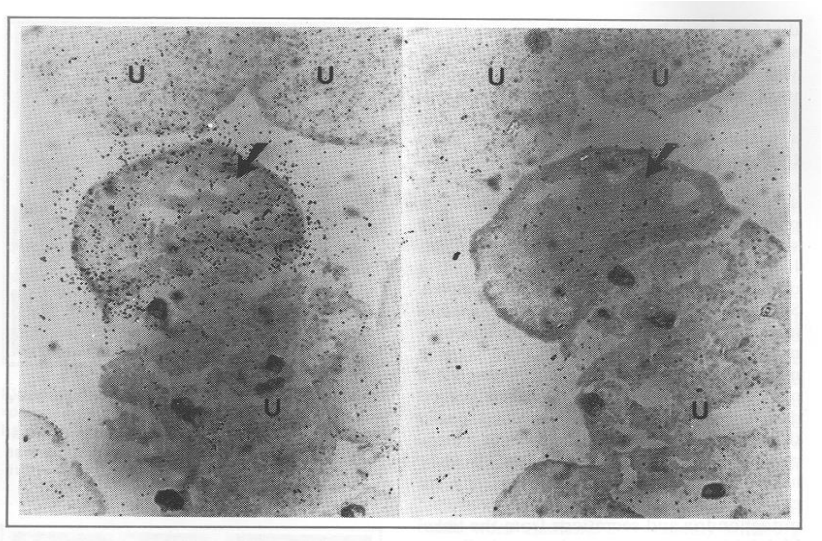

Figure 7. *Detection of anti-sporozoite antibodies in hybridoma culture fluids by immuno-autoradiography: autoradiograph of an infected acinus (*arrow*) in sequential sections through an infected salivary gland, incubated with either anti-sporozoite (*left*) or control (*right*) antiserum. Uninfected acini (U) are not labeled. Salivary glands from* R appendiculatus *ticks, heavily infected with* T p parva *sporozoites, were collected and 5 µm cryostat sections prepared, mounted on slides, fixed in 0.25% glutaraldehyde for 5 min and stored at –20°C. Sections were thawed, washed once with phosphate-buffered saline (pH 7.4) containing 1 % bovine serum*

albumin and 0.05% sodium azide (washing buffer-WB), incubated for 45 min with 20 µl of the culture supernatant being tested for the presence of monoclonal antibody, and washed 3 times with WB. Sections were then incubated for a further 45 min with 20 µl of a mixture of ^{125} 1-labeled goat anti-mouse IgG and IgM (2×10^4 cpm for each), washed 3 times with WB and dried. To demonstrate parasite DNA inside the infected salivary gland acini, preparations were fixed at. room temperature for 1 h with 5N HCI and stained for 1 h with Schiff 's reagent: The slides were then dipped in photographic emulsion (Ilford L4) and left to dry and expose overnight. Preparations were then developed, fixed and examined microscopically. The presence of silver grains over an infected acinus indicates specific binding of the antibody to sporozoite antigen within the acinus.

This assay system was used to screen 360 monoclonal cell lines, and 5 were identified which showed strong antibody activity to *T p parva* Muguga sporozoites. One IgG_3 antibody, $MAbD_1$ was selected for further neutralization studies. In vitro experiments showed that this antibody neutralizes sporozoites of *T p parva* Muguga, *T p parva* Uganda and two strains isolated from the Kenya coast, *T p parva* Mavuen*i* and *T p parva* Marikebuni. When sporozoites of these strains were incubated with the $MAbD_1$ antibody diluted at a rate of 1:1000, they were inhibited from infecting bovine peripheral blood leucocytes (PBL), whereas bovine PBL were infected by sporozoites incubated with a control antibody ($MabV_3$), as shown in Figure 8. The activity of this antibody was confirmed by in vivo tests: four out of six cattle inoculated with *T p parva* Muguga sporozoites treated with different concentrations of the $MAbD_1$ antibody did not become infected, while two others became infected but recovered. Animals inoculated with sporozoites treated with a control antibody all died.

Isolate	Antibody Dilution	% Wells showing Cell line establishment	
		$MAbD_1$	$MAbV_3$
T p parva Muguga	10^{-1}	0	100
	10^{-2}	0	100
	10^{-3}	0	100
	10^{-4}	100	–*
T p parva Uganda	10^{-1}	0	100
	10^{-2}	0	–*
	10^{-3}	0	–*
	10^{-4}	0	–*

T p parva Mavueni	10^{-1}	0	96
	10^{-2}	0	–*
	10^{-3}	0	–*
	10^{-4}	0	–*
T p parva Marikebuni	10^{-1}	12	100
	10^{-2}	0	–*
	10^{-3}	8	–*
	10^{-4}	96	–*

*Test not done.

Figure 8. *Inhibition of* Theileria *infection in bovine PBL by the $MabD_1$ monoclonal antibody and a control antibody ($MabV_3$). Fresh sporozoites were prepared from* R appendiculatus *salivary glands infected with* T p parva *stocks. Glands were dissected out under aseptic conditions and homogenized in a tissue grinder in complete L15 medium. After centrifugation at 50 g for 5 min, the supernatant was collected and adjusted to an equivalent of 100 ticks/m^{-1}. Twenty µl of $MAbD_1$ diluted in L15 were added to 20 µl of sporozoite preparation to give final antibody dilutions of 10^{-1},10^{-2}, 10^{-3}, or 10^{-4}. After 30 min incubation on ice, 5 µl of undiluted rabbit complement (C') was added and the mixture incubated a further 30 min at 37° C. Where appropriate, the monoclonal antibody $MAbD_1$ was substituted by complete L15 or by the control antibody $MAbV_3$. After incubation with antibody and C', 2.5×10^5 PBL isolated on Ficoll-Hypaque (in 10 µl of complete L15) were mixed with the sporozoite preparations and left for 1 h at room temperature. The cells were then washed, resuspended in 2.5 ml complete L15, and 100 µl aliquots dispensed onto a feeder layer derived from fetal bovine thymus in 24 wells of a flat bottomed 96-well microtiter plate. The final volume in each well was 200 µl and the medium was replenished every 3 days by replacing 100 µl with fresh complete L15. Plates were examined weekly for 3 to 5 weeks and the number of wells with cell growth was determined.*

These results and those of the first study suggest that protective immunity against *Theileria* infections may involve the production of specific antibodies. Having shown that anti-sporozoite

antibodies can neutralize *Theileria* sporozoites in vitro, ILRAD scientists are now investigating their possible role in protecting cattle from infection. Both 1982 studies also suggest that strains of *T p parva* isolated from widely separated locations may share a common antigen which might be used as the basis for an effective vaccine.

Further work is in progress to isolate and characterize the sporozoite antigens which generate a protective immune response. These antigens are probably proteins, but their chemical nature is being investigated to rule out the possibility that they might be carbohydrates or lipids. Sporozoite antigens which are likely to be proteins are being identified according to their molecular weight using immunoautoradiographic techniques. One protein has been identified with a molecular weight of about 105,000 daltons which is specific to *Theileria* sporozoites. If the relevant antigen(s) can be precisely characterized, appropriate DNA could possibly be cloned in bacteria, using recombinant DNA technology, or artificially synthesized to produce sufficient quantities for use in immunization trials.

Development of a vaccine based on schizont-infected cells

Cattle can be immunized experimentally against ECF by inoculating them with bovine lymph cells which are infected with *Theileria* macroschizonts. For successful immunization to occur, the macroschizonts must leave the foreign cells and somehow enter and transform the host's own cells. Before this can happen, the animal's immune system tends to reject the foreign lymphoid cells. This problem can be overcome by taking cells from the animal to be immunized, infecting them with sporozoites in vitro and injecting them back into the same animal. Another approach is to use very large numbers of infected foreign lymphoid cells (about 100 million) so that at least a few survive the initial rejection effect. However, neither of these approaches is fully safe or practicable for widespread application. A full understanding of the way macroschizonts transfer between cells and the associated host responses could lead to an improved vaccine based on schizont-infected cells or other measures which would prevent or modify the effects of the disease.

In clinical cases of ECF, infected cells stimulate other cells to multiply and to kill surrounding cells. Changes on the surface of the parasitized cells are thought to trigger these different responses. Scientists at ILRAD are investigating precisely what types of cells are infected by *Theileria* sporozoites, what changes occur on these cells, how these changes are induced by the macroschizont, and what responses they stimulate in other cells.

Researchers elsewhere have shown that vertebrate cells have genes whose products regulate cell proliferation, some inducing and others inhibiting growth. Tyrosine protein kinases induce cell growth and proliferation, while a protein phosphatases inhibit growth, producing a normal balance. The uncontrolled growth of *Theileria*-infected cells can be viewed as a breakdown of these normal homeostatic controls: the *Theileria macroschizont* may produce or induce the production of excessive amounts of tyrosine protein kinase or inhibit the synthesis or function of protein phosphatases which control the growth process.

A variety of techniques and assay systems are being developed at ILRAD to investigate these possibilities. The products of cells parasitized with *T p parva* and *T p lawrencei* are being

screened to identify growth-promoting factors as well as toxic products which eventually lead to cell destruction.

Other studies carried out in 1982 concentrated on the types of cells which are infected by *Theileria parasites*. Scientists raised monoclonal antibodies which recognize specific types of bovine lymph cells. These were used to examine how many bovine cell types can be infected in vitro by different strains of *T p parva* sporozoites and which types are transformed into enlarged blast cells containing parasite macroschizonts. This investigation has shown that some B-cells, several subpopulation of T-cells and at least one monocyte population can be infected and transformed by the parasite in vitro. However, preliminary studies indicate that fewer cell types are actually involved in lethal infections in cattle. These observations suggest that *Theileria* parasites may infect many cell types in cattle but the host may control the proliferation of some cell types while other types are uncontrollable.

Studies are now in progress to determine what makes one infected cell type less controllable than another. Scientists are also investigating whether the cells which become uncontrollable in different animals display common antigens when the animals are infected with the same *Theileria* strain and whether different *Theileria* strains infect and transform different cell types.

Earlier studies indicated that parasitized cells are not induced to proliferate by a simple early event, but rather that the continuous presence of a *Theileria* macroschizont is required to maintain the proliferative state. As host cells are stimulated by *Theileria* macroschizonts to divide, some cells are produced which are free of parasites. Comparative studies of parasitized and non-parasitized daughter cells are being carried out to determine the factors and metabolic pathways involved in the maintenance of the parasitized state. When parasitized cells are cloned in vitro, a consistent proportion of parasite-free daughter cells is produced, ranging from 1 to 15% for different cell lines. These non-infected cells are viable, though they appear to stop dividing or divide at a much lower rate than the infected cells. Evidence suggests that non-parasitized cells originate from abnormal cell divisions with the *Theileria* macroschizont nuclei all transferred to one daughter cell.

The terminal stage of ECF is characterized by massive cell destruction, but there is also evidence that cytotoxic (cell-killing) responses are involved in the acquisition of immunity. The different between the two situations appears to lie in the specificity of the response. In fatal cases of ECF, an animal generates cytotoxic cells which indiscriminately kill its own parasitized and non-parasitized cells: these cytotoxic cells are also capable of killing parasitized and non-parasitized cells from other animals. By contrast, if an animal has acquired immunity to a particular strain of the parasite and is rechallenged with the same or similar strain, cytotoxic cells are generated which kill only parasitized cells of that animal, thus ridding it of infection.

The specificity of the cytotoxic response in immune cattle probably involves recognition of major histocompatibility complex MHC antigens. In other animal species, these antigens act as targets for cytotoxic responses to virus-infected or foreign cells. Researchers elsewhere have identified a series of class 1 MHC antigens in cattle. During the past year, a study was carried out in collaboration with the Veterinary Research. Department of the Kenya Agricultural Research Institute which demonstrated clearly that these MHC antigens act as targets for cytotoxic cells

(Figure 9). Further studies are in progress to determine whether specific killing of *Theileria*-infected cells by cytotoxic cells also involves recognition of these MHC antigens.

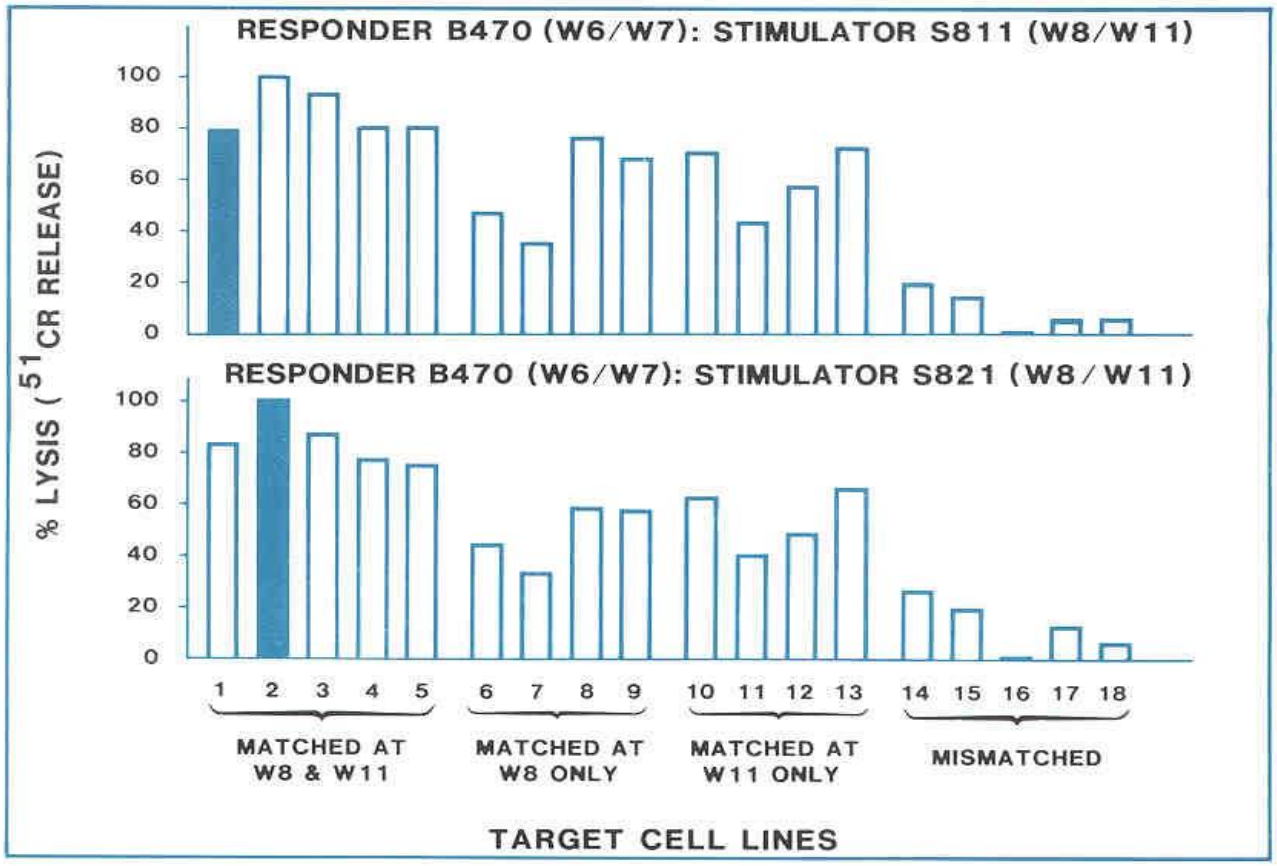

Figure 9. *Bar charts showing levels of cytotoxicity generated in an allogeneic mixed leucocyte reaction (MLR) using responder bovine PBL from one animal (B470) with stimulator PBL from two different animals (S811 and S812). Cytotoxicity levels were measured in a ^{51} Cr release assay on a series of target cell lines which were matched (columns 1–5), semimatched (columns 6–13) or mismatched (columns 14–18) with the stimulator cells with respect to class 1 antigens coded by the A locus of the bovine MHC. The level of cytotoxicity attained closely correlated with the degree of sharing of these MHC determinants. Thus, the level of cytotoxicity was high not only on the cells of the animals used to stimulate the response (solid columns), but also on cells fully matched with the stimulator. Very low levels of cytotoxicity were obtained on mismatched targets, while the level of cytotoxicity on semimatched targets was intermediate.*

Another study was carried out to identify specific antigens on the surface of *Theileria* infected cells. First, cattle cells were infected in vitro with *T p lawrencei* sporozoites of a strain originally isolated from a buffalo. A monoclonal antibody (J7) was raised against these cells and was found to recognize an antigen on the surface of some of the cells, but not others. The antigen was not present on any non-infected cells. Using the fluorescence-activated cell sorter (FACS) and monoclonal antibodies available at ILRAD to distinguish different populations of bovine cells, it was shown that the cells which express the antigen are all of a particular type. Further studies

have shown that the antigen is not produced by the parasite itself, but is probably induced on one type of host cell type by the presence of the parasite.

It may be possible to immunize animals with non-infective preparations of parasitized cells which will stimulate the specific cytotoxic responses that appear to confer immunity. Two approaches were investigated in 1982. In one study, cattle were immunized with cell membrane preparations made from infected cell lines. Three out of four animals immunized with preparations from their own cells proved immune to a lethal dose of *T p parva* sporozoites, whereas three animals inoculated with preparations from the cells of other animals were fully susceptible. The finding that some animals can be immunized with cell membranes prepared from their own *Theileria*-infected cells supports the hypothesis that protective immune responses are directed against antigens on the cell membrane and that such responses are restricted by the host genotype.

In further experiments, the responses of bovine lymphocytes to infected cell membranes were monitored to determine whether proliferative responses in vitro reflect an animal's immune status. When membranes from infected cells were added to lymphocytes taken from the same animal, they did not stimulate any proliferation, either in naive or immune cattle. However, if the membranes were added to macrophages (a cell type which presents antigens to lymphocytes) and then mixed with lymphocytes, they sometimes stimulated a strong proliferative response. Proliferation was observed in cells from cattle which had been immunized with membrane preparations, but not in cells from cattle which had been immunized by infection with sporozoites and treatment. A proliferative response was also observed in one non-immune animal which had been injected with irradiated *Theileria*-infected cells. These findings suggest that the antigen on the cell membrane which stimulates a proliferative response is present only on infected cells cultured in vitro, and is therefore probably not involved in stimulating protective immunity in cattle.

Cattle have also been inoculated experimentally with intact cells grown in vitro and rendered non-infective by irradiation. Large doses of irradiation were required to completely neutralize the infectivity of the cells. Cattle inoculated with these cells showed some cytotoxic responses when challenged with sporozoites but still became infected. The failure to achieve immunity despite the detection of cytotoxic responses could suggest that other responses may be required for protective immunity, that the cytotoxic responses measured are directed against a number of different antigens, not all of which are relevant, or that the specific host cell types infected in vitro are different from the cell types infected by challenge with sporozoites.

Epidemiology and experimental ECF control

Cattle inoculated with *T p parva* sporozoites and treated stimultaneously with a long-lasting oxytetracycline antibiotic (Terramycin LA-Pfizer) often develop no or only mild disease symptoms and subsequently resist infection by the same strain of the parasite. This 'infection and treatment' approach to immunization has several disadvantages, but it offers the most immediate possibility of improved disease control while work continues on the development of a safe and effective vaccine.

One disadvantage is that different strains of *T p parva* are often not cross-protective: immunity conferred against one strain will not necessarily ensure protection against another. Secondly, not all strains can be controlled by oxytetracycline, so the initial infection with live parasites may be fatal. Finally, buffalo are often chronic carriers of ECF, and in areas where cattle come into contact with buffalo the pattern of parasite strains is often very complex, making control more difficult.

Epidemiological studies at ILRAD focus on all these problems. The aim is to investigate and introduce improved control methods in restricted locations on a trial basis with the possibility of expanding over wider areas if the initial trials prove successful.

Strain Characterizations

The identification and classification of *Theileria* strains isolated in the field is an important component of ILRAD's epidemiology program. Until recently, the only way to identify specific strains of *T p parva* was by cross-immunity trials in cattle. These are expensive and imprecise. Now monoclonal antibodies are raised at ILRAD which can be used to screen macroschizont-infected cells and identify parasite strains.

By the end of 1982, a battery of 16 anti-macroschizont monoclonal antibodies had been raised and tested against 22 stocks of *T p parva* isolated from widely separated areas of Kenya, Uganda, Malawi and Zambia. The activities of the different monoclonal antibodies were measured using the indirect fluorescent antibody (IFA) test. Based on comparisons of these responses, all the stocks tested fell into one of three groups, as shown in Figure 10. The pattern of reactions remained the same after the stocks were passed through ticks or cattle. The groupings do not correlate with the geographic areas from which the stocks were obtained.

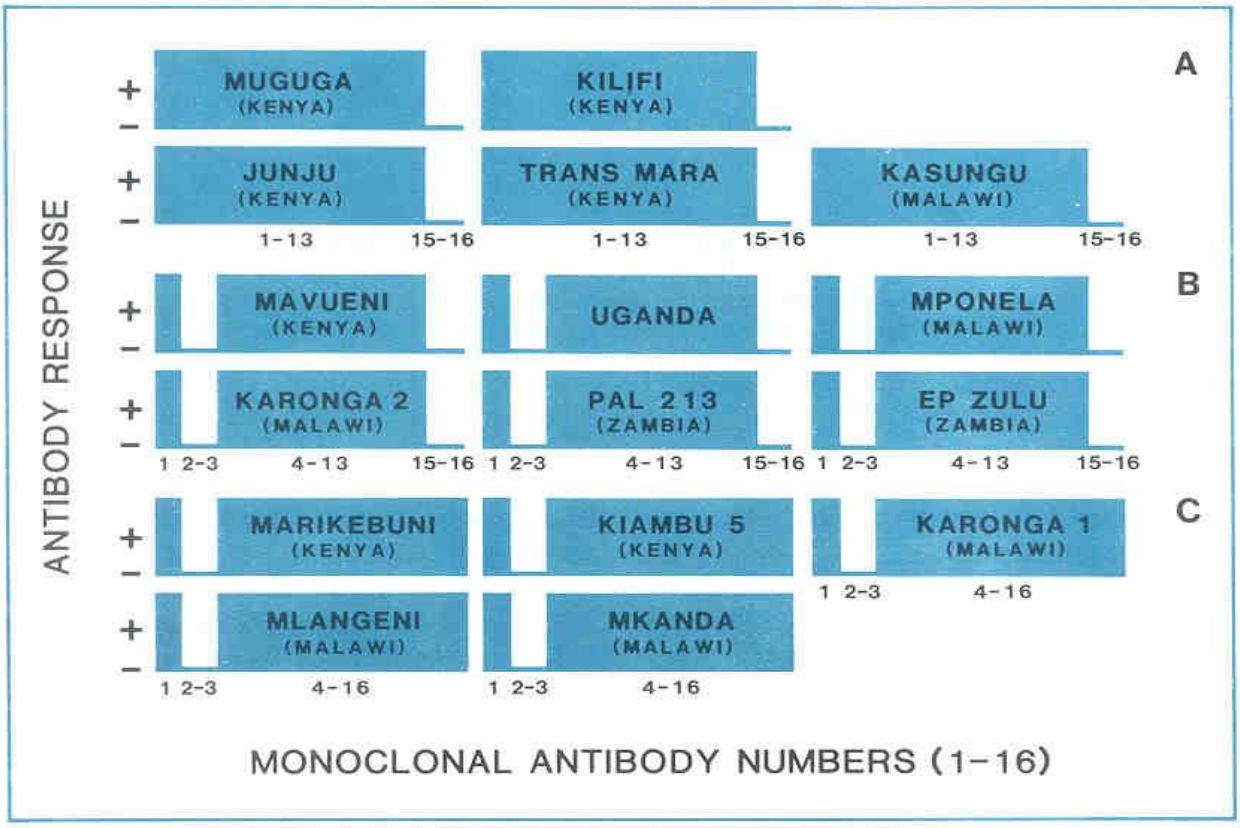

Figure 10. *Responses of 16 monoclonal antibodies to macroschizonts of 16* T p parva *stocks, measured by the IFA test. A total of 23 stocks from Kenya, Uganda, Malawi and Zambia have been screened. They all appear to fall into one of three profile groups (A, B and C), irrespective of geographic origin.*

The best material for obtaining profiles of monoclonal antibody reactions proved to be macroschizont-infected lymphoid cells taken from infected cattle and cultured in the laboratory. Profiles can also be obtained within hours from biopsy material taken from infected animals; this technique could be important in planning an immunization strategy to control a disease outbreak in the field. The reactions of monoclonal antibodies have also been measured experimentally by immuno-peroxidase reaction procedures, but this alternative approach has not yet been thoroughly evaluated.

Cross-immunity trials were carried out in cattle to test the accuracy of the monoclonal profiles in characterizing disease stocks. Cattle were immunized experimentally against one *Theileria* stock and rechallenged with the same stock. Then they were challenged with another stock of the same antibody profile group or with a stock of a different profile group. The results are summarized in Figure 11. Animals challenged with the same stock as their original infection or with a stock of the same profile group showed very mild disease symptoms or none at all. By contrast, animals challenged with a stock of a different profile group often showed severe symptoms and some died.

Type of challenge	None	Level of reaction (%)			Number of cattle
		Moderate	Severe	Death	
Same stock	83	17	0	0	53
Different stock, same profile group					
	75	25	0	0	16
Different stock, different profile group					
	38	28	12	22	49
Controls	0	3	23	74	34

Figure 11. *Reactions of cattle immunized experimentally against an ECF stock and challenged with the same stock or with a different stock from the same or another monoclonal antibody profile group. These results suggest that antibody profiles of* T p parva *isolates reflect cross-protective patterns.*

Future work will concentrate on further correlation between monoclonal antibody reactions to different *Theileria* stocks and cross-protection in cattle. Monoclonal antibodies will also be used as markers in hybridization experiments. It may be possible to develop hybrid *Theileria* strains as the basis of a vaccine which will protect animals over a wide geographic area.

Immunization trials

Previous workers have immunized cattle against ECF by 'infection and treatment' with some success in field trials carried out in Kenya, Tanzania and Rwanda. In cooperation with the Kenya Ministry of Livestock Development and Agricultural Development Corporation, ILRAD scientists are examining this method in three areas at the Kenya coast where livestock management is good and the epidemiology of the disease is well understood.

Several *T p parva* isolates were made from different sites at the coast, and stocks of each isolate have been produced and characterized according to their monoclonal antibody profiles. *T p parva* Kilifi and *T p parva* Marikebuni were selected as key stocks for immunization trials. Animals are immunized by injecting them with sporozoite material and treating them simultaneously with Terramycin.

Scientists are investigating the earliest age at which calves can be safely immunized. This is an important consideration because the immunization of young animals results in early protection and lower costs. Also following ECF immunization, calves can be exposed to babesiosis, heartwater and anaplasmosis in the field. In this way, young animals can acquire immunity to

these other tickborne diseases while they still retain a level of maternal resistance, whereas such a procedure would be hazardous for older animals.

In one study, 2-week-old Boran calves were immunized at ILRAD as safely and effectively as older cattle. In a related experiment, 6-month-old calves were immunized and afterwards their weight gains were compared with those of untreated controls. Figure 12 shows that growth rates in the immunized calves were not affected either by the initial immunization or by subsequent disease challenge.

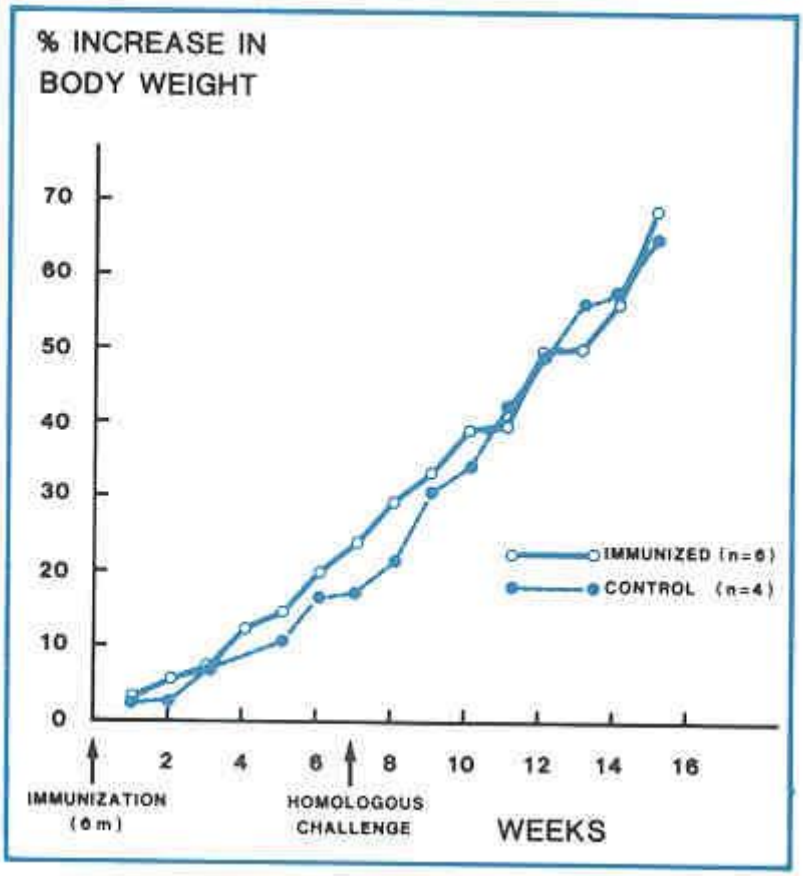

Figure 12. *Weight gains over 4 months of calves immunized against ECF at 6 months of age and challenged 7 weeks later with the same disease strain. Weight gains throughout the period were comparable to those of controls.*

Techniques are being developed to measure doses of sporozoite material more precisely. Other aspects of experimental immunization under study include the mechanisms of oxytetracycline action and the possibility of using other drugs or techniques for containing sporozoite-induced infections. This work has shown that if the dose of parasites is too high oxytetracycline may not be effective in containing the ensuing reaction and further treatment, such as with parvaquone (Clexon-Wellcome), may be necessary. However, when a lower dose of parasites is used, calves undergo a mild or inapparent reaction and are fully resistant to subsequent lethal challenge.

This method is being examined under field conditions at three sites at the Kenya coast. A group of 18-month-old Boran cattle was immunized at ILRAD and transported to Mariakani with control animals. At Mtwapa, a group of Jersey calves was purchased and immunized locally. The third trial is being conducted at Malindi, where a group of Sahiwal/Red Poll cross calves has been immunized. When a single dose of oxytetracycline was administered to this third group, some calves needed additional treatment with parvaquone, but when oxytetracycline was given to the group at Mtwapa at the time of infection and again 5 days later, no adverse reactions were recorded. The response of the three cattle groups to field challenge is now awaited.

If these experiments are successful, they will demonstrate that stocks of *T p parva* can be isolated, characterized and used to immunize cattle against local strains. The next step will be to extend similar trials to other areas in Kenya and to other countries.

Theileria studies in wildlife

African buffalo *(Syncerus caffer)* are chronic carriers of *T p lawrencei* which can cause high mortality in cattle. Figure 13 shows the distribution of cattle and buffalo in East Africa. In areas where cattle come into contact with buffalo, the pattern of *Theileria* strains becomes extremely complex and a breakdown in immunity can occur. In cooperation with the Kenya Government's Veterinary Research Laboratories, scientists at ILRAD are studying *Theileria* in wild animals and its transmission to domestic cattle.

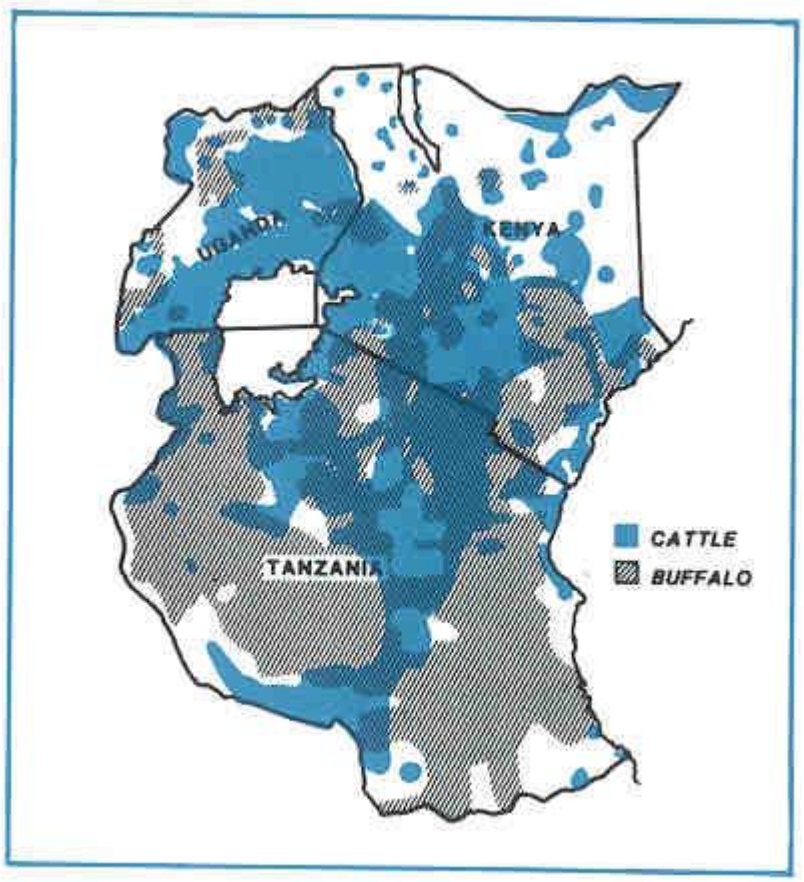

Figure 13. *Distribution of buffalo* (Syncerus caffer) *and cattle in East Africa.*

Work is in progress to define more precisely the relationship between *T p lawrencei* and *T p parva.* These two *Theileria* subspecies cannot be distinguished serologically, though there are indications that they may invade different types of cells. There is also some evidence that *Theileria* parasites carried by buffalo change antigenically over time. This would have serious consequences for attempts to immunize cattle against locally isolated stocks of ECF in areas where buffalo are present. To investigate this possibility, attempts are being made to infect a buffalo with a clone of *T p lawrencei* and to isolate parasitized cell lines from the animal sequentially for antigenic analysis.

Aspects of tick biology and *Theileria* transmission

It has been demonstrated that livestock and laboratory animals acquire resistance to tick infestation. Antigens secreted by the tick during feeding appear to stimulate a delayed-type hypersensitivity inflammatory response in the skin which makes the bite site unfavourable for the feeding process. In collaboration with ICIPE, scientists have analysed serum from rabbits resistant to *R appendiculatus* ticks using crossed immune electrophoresis in order to identify tick antigens which stimulate the hypersensitivity reaction. One promising antigen has been selected and is now being purified from tick salivary glands for further study.

Research has also suggested that tick products may stimulate an immune response in the mammalian host which reduces the viability of eggs laid by the ticks after feeding. Rabbits were immunized with an extract of fed adult female ticks and scientists used crossed immune electrophoresis to identify 10 to 12 tick antigens recognized by the rabbits' sera. Antibodies were generated against four of these antigens and were used successfully to transfer the adverse effect on egg viability to feeding ticks. Precipitin line immunization will now be used to identify the specific antigen responsible for this effect and to analyse its synthesis and function. Eventually, this antigen or the antigen which stimulates resistance to tick bites may be used in experimental immunization trials.

It has been hypothesized that tick saliva also contains substances which inhibit the immune responses of cattle, making them more susceptible to *Theileria* infection. Tick salivary gland extracts (TSGE) and saliva from infected and uninfected ticks have been screened for enzymes, enzyme inhibitors and anticoagulants which could play a role in circumventing host defences against tick infestation and transmission of infection. It has been shown that tick saliva and TSGE have a histamine inactivating enzyme and inhibitors of chymotrypsin and thrombin. Very little difference was found between infected and uninfected TSGE, indicating that the sporozoite contribution to these functions is minimal. Work is now in progress to purify some of the TSGE proteins and peptides. Eventually, cattle will be immunized with these proteins and assessed for any increased capacity to control tick infestation and ECF transmission.

Earlier work at ILRAD indicated that *Theileria* sporozoites stimulate bovine lymphocytes to proliferate much more readily if the bovine cells are already multiplying in response to the mitogen Concanavalin A. This finding suggests that substances in tick saliva which stimulate a proliferative response may also make bovine lymphoid cells more receptive to sporozoite invasion. The stimulatory effect of TSGE on bovine leucocytes was tested in vitro, but so far result, have been inconclusive.

Trypanosomiasis

Trypanosomiasis is a serious, often fatal, parasitic disease which occurs in large areas of Africa, Latin America, the Middle East and Asia. It affects most species of domestic livestock, many types of wild animals, and man. In Africa, trypanosomiasis is found over approximately one-third of the continent, as shown in Figure 14.

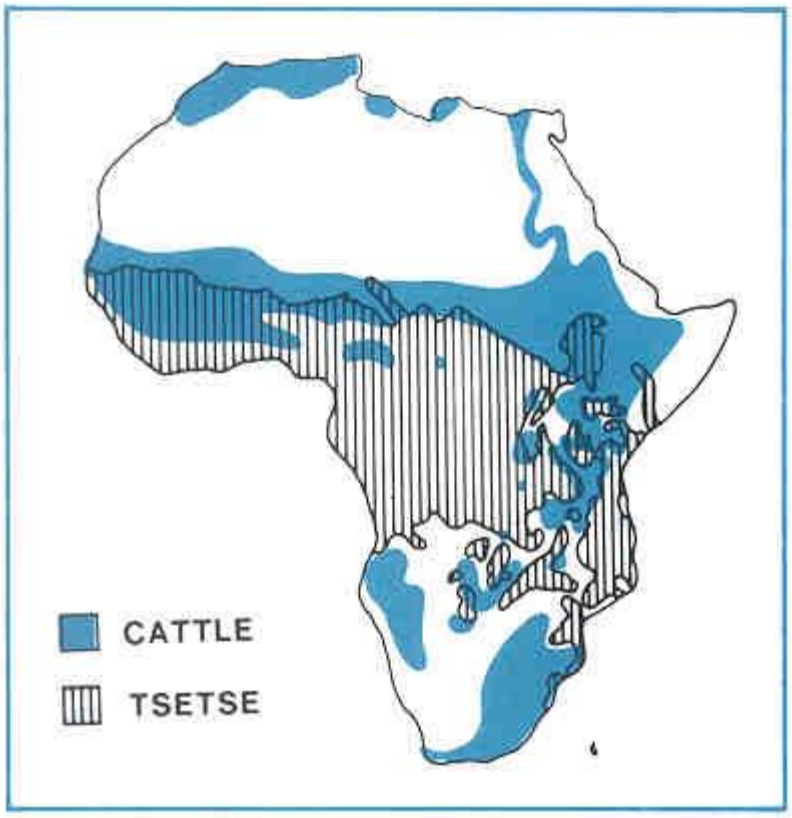

Figure 14. *Major cattle-production areas and tsetse-infested zones in Africa. There is little overlap except in the regions of West and Central Africa where trypanotolerant N'Darna and West African Shorthorn cattle are kept.*

Trypanosomiasis is caused by single-celled parasites which primarily invade the bloodstream. Different species of trypanosome infect different host species. The most important trypanosomes in economic terms are the tsetse-transmitted species which infect cattle, sheep and goats in Africa—*Trypanosoma congolense, T vivax* and *T brucei* sspp. Trypanosomiasis research at ILRAD focuses on these three species.

In Africa, trypanosomes are usually transmitted by tsetse flies *(Glossina spp)* and occasionally by other biting flies. About 30 species and subspecies of tsetse are found in Africa. All of these are capable of transmitting trypanosomiasis, but some are more important vectors than others. In many parts of the world, trypanosomiasis is transmitted by other biting flies or occasionally

sexually. Trypanosome species which are not transmitted by tsetse flies, such as *T evansi* cause significant livestock disease in Africa, Asia and certain areas of Latin America.

Figure 15 summarizes the lifecycle of the three main African trypanosome species. The parasites are ingested by tsetse flies when they feed on the blood of infected animals. They undergo a cycle of development in various locations within the fly depending on the trypanosome species. All three species finish this phase of their development as metacyclic forms in the tsetse mouth parts or salivary glands.

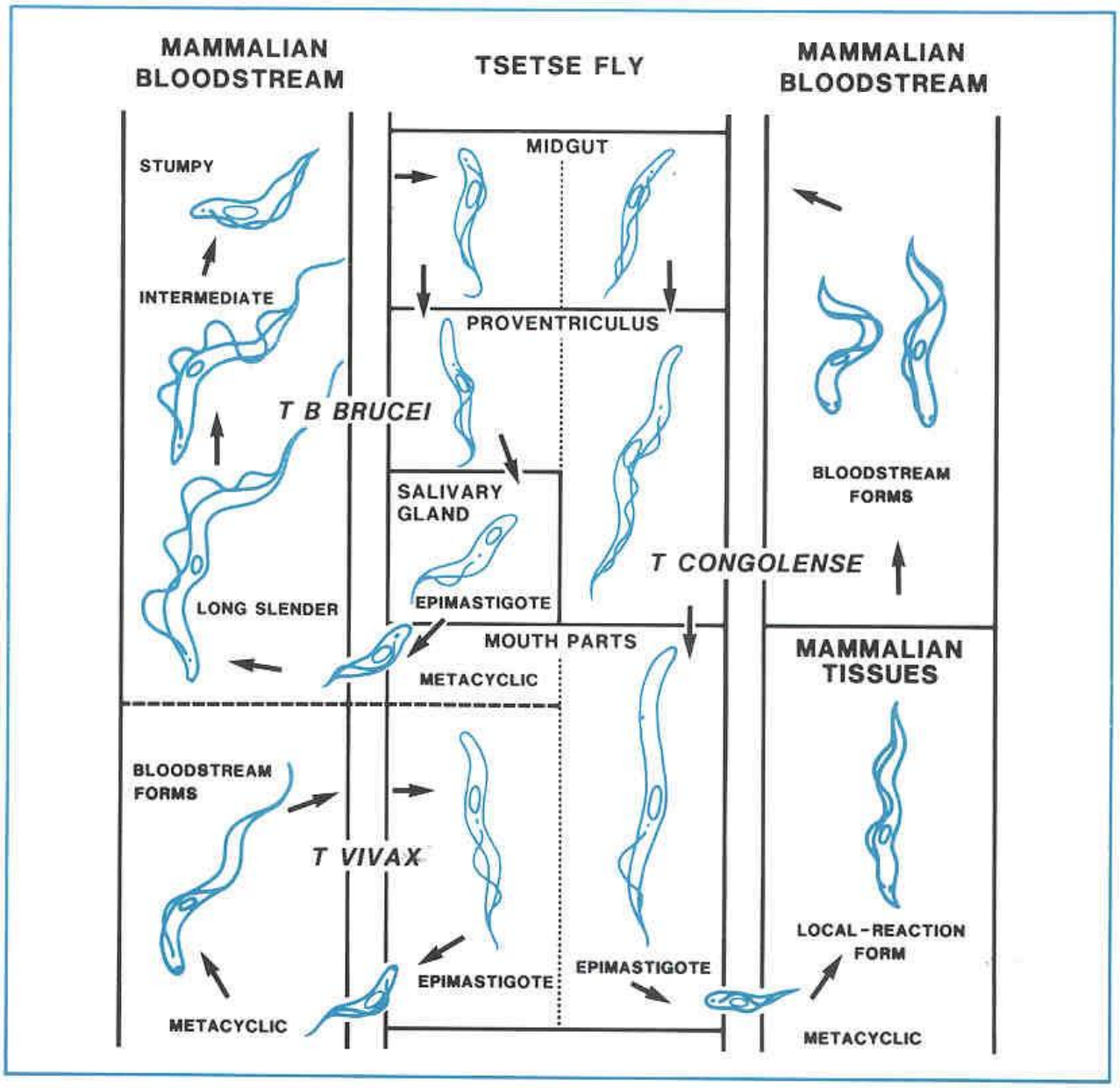

Figure 15. *Life cycle of* T b brucei, T congolense *and* T vivax. *Heavy outlines indicate parasite forms with surface coats consisting of variable glycoprotein antigens. Light outlines indicate uncoated forms which are not infective to mammals.* T b brucei *develops in the tsetse midgut, proventriculus and salivary glands, where metacyclic forms occur which are infective to mammals.* T congolense *develops in the tsetse midgut, proventriculus and mouth parts, where infective metacyclic forms are produced.* T vivax *develops in the tsetse mouthparts.*

The metacyclic trypanosomes are transmitted into an animal's skin when an infected tsetse feeds. In the case of *T b brucei* and *T congolense*, a prominent swelling, called a chancre, develops at the site where the parasites are introduced. The trypanosomes develop in the chancre: in *T congolense* infections a local reaction form can be distinguished at this stage. The parasites then invade the local lymph vessels and move into the bloodstream where they undergo further development. The infection which follows is characterized by successive waves of parasitaemia, as parasite populations multiply rapidly and die off. *T vivax* and *T b brucei* parasites may also invade the connective tissues, and, in later stages of the disease, *T b brucei* may be found in the central nervous system. In the human disease, African sleeping sickness, *T b rhodesiense* or *T b gambiense* parasites invade the central nervous system, causing death.

Trypanosomiasis in livestock causes anaemia, poor growth, infertility, and abortion. Many infected animals die. Others may eventually eliminate the parasites and recover, but their growth and productivity are often reduced.

At present, trypanosomiasis is controlled primarily by spraying tsetse resting and breeding sites with insecticide and by treating livestock regularly with trypanocidal drugs. However, neither of these approaches is completely effective in areas of heavy infestation. Further problems include the repeated use of the few drugs available, which may lead to drug resistance, and the possibility of environmental pollution due to large-scale insecticide spraying.

The goal of ILRAD's trypanosomiasis program is to develop effective, safe and economic methods to control the disease. This could be by immunological, chemical or genetic means. Research concentrates on the development of control measures which interfere with the parasites themselves or improve the responses of infected animals. Scientists are also studying aspects of trypanosomiasis epidemiology at a field site near the Kenya coast and in a collaborative animal production research network at sites in several African countries.

Towards improved control of the parasites

In the course of an infection, successive generations of trypanosomes display different antigens on their surface coats. These antigens are variable surface glycoproteins (VSGs). Trypanosomes with different VSGs are called variable antigen types (VATs). Trypanosomes in the tsetse fly are not coated with variable antigens until they develop into the metacyclic forms which are transmitted to mammals. Metacyclic trypanosomes are thought to display a limited number of VSGs, while the parasites which develop in the bloodstream may display very large numbers, thus evading the immune responses of the host.

In 1982, research on trypanosomes concentrated on the genetic basis of antigenic variation and on the structure and synthesis of the variable antigens. Other studies focused on the identification of specific variable antigens and on their role in the course of infection. Techniques developed earlier to cultivate and maintain trypanosomes in vitro were extended as progress continued towards the goal of maintaining all three major trypanosome species on a continuous basis. This technology has made it possible to study the different stages of the trypanosome lifecycle in detail as well as the mechanisms involved in the transition from one stage to the next. The goal of all these projects is to identify factors which could be manipulated to disrupt parasite development.

Antigenic variation: the genetic basis

Antigenic variation involves switching on and off the expression of a large family of genes which code for different VSGs. Molecular biologists at ILRAD are studying the changes which occur when different genes are expressed. This work is being carried out in collaboration with Cambridge University's Molteno Institute, the Université Libre de Bruxelles and the Vrije Universiteit Brussel.

The expression of different VSGs during the course of infection is associated with rearrangements in the parasite DNA. Two types of gene rearrangement have been observed in *T b brucei.* Sometimes the expression of a VSG gene is accompanied by the appearance of a duplicated copy of the gene at a new location in the genome. The extra copy disappears when the gene is no longer expressed. This type of rearrangement has been termed 'expression-linked duplication'. When scientists at ILRAD cloned parasites from one *T b brucei* stock at different times, they also found three different VSG genes which were not duplicated when their antigens were expressed. This indicates that a second type of gene rearrangement can occur in association with antigenic variation. The different type of gene rearrangement is related to the specific VSG gene being expressed, not to general differences between typanosome stocks.

Scientists have been studying four VSG genes from the ILTaR 1 antigen repertoire. Of these, only one (ILTat 1.1) undergoes rearrangement involving expression-linked duplication. There are four distinct copies of the ILTat 1.3 gene and three of the ILTat 1.4 gene in each member of a closely related group of trypanosome clones, and the number of copies of these genes does not vary with expression. One copy of the ILTat 1.3 gene has been characterized in detail and compared with known ILTat 1.3 messenger RNA. Beyond a homologous region, the two sequences differ completely, indicating that this copy gene cannot code for a normal VSG.

Rearrangements have been detected in the genomic environment of some, but not all, copies of these two genes. For those copies which do undergo rearrangement, ILRAD scientists have found that clusters of enzymes cut the DNA at a site which is probably at the end of a chromosome. Figure 16 shows eight restriction enzyme fragments of DNA during a period of treatment with the enzyme Bal 31 exonuclease. Two fragments (12.0 and 7.2 kilobases) have been digested by the enzyme, while the others have not. This indicates a natural double-strand break in the DNA at this site.

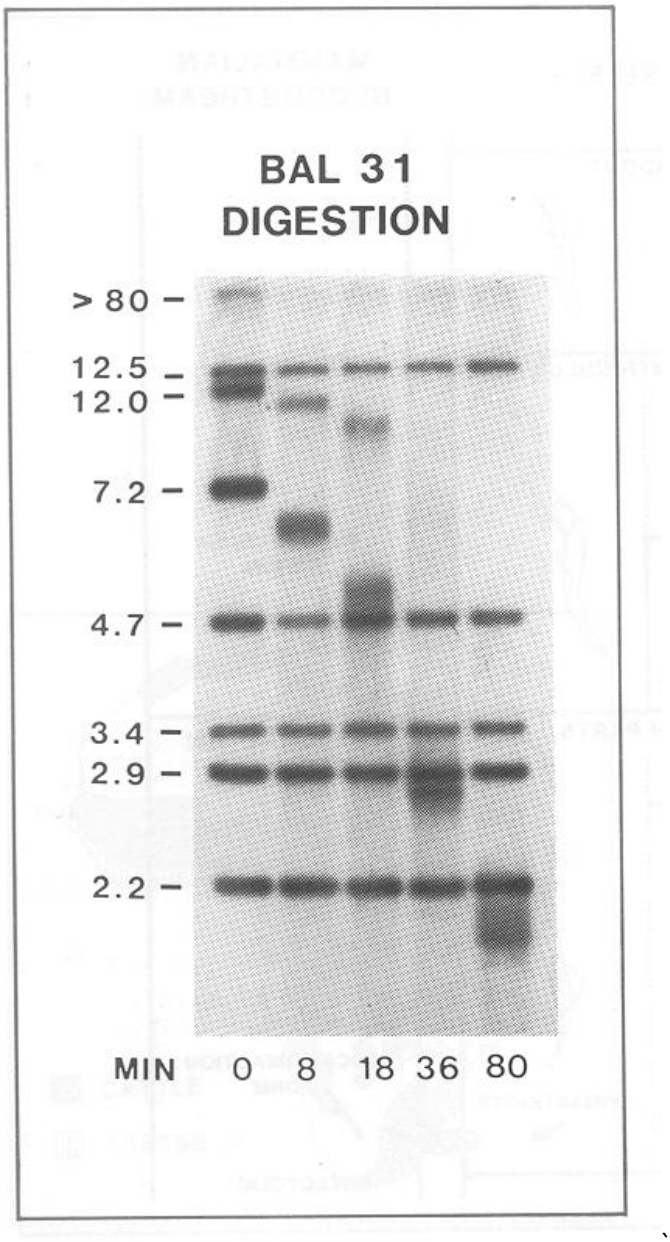

Figure 16. *Bal 31 digestion of* T b brucei *nuclear DNA: 40 µg of DNA was digested with 13 units of Bal 31 enzyme in 600 mM NaCl, 12mM $CaCl_2$,, 12 mM $MgCl_2$, 20 mM Tris-HC1 pH 8 and 1 mM EDTA. The reaction was incubated at 30°C and aliquots were taken at times shown by pipetting into water-saturated phenol. Each DNA was precipitated with ethanol and subsequently digested with Aval restriction endonuclease. The digested DNAs were electrophoresed in a 0.8% agarose gel and transferred to nitrocellulose paper. The plasmid pcBC1 containing the complete coding sequence for VSG ILTat 1.3 was nick-translated and hybridized. The figure shows the results of the blotting experiments: only fragments 12.0 and 7.2 kilobases were digested by the enzyme.*

Thus two copies of the ILTat 1.3 gene are both adjacent to the end of a DNA molcule. These copies might be located on unusual extra chromosomal DNA structures. To test this possibility, DNA was prepared from three trypanosome clones expressing VSGs ILTat 1.2, 1.3 and 1.4 and fractionated on sucrose density gradients. Figure 17 shows the distribution of the four copies of the ILTat 1.3 gene in one of the gradients. Copy D is located on fractions 16 and 17 and has been clearly separated from the bulk of the nuclear DNA. It appears that this copy is located on a minichromosome—a DNA molecule that is separate from, and smaller than, the bulk of the trypanosome DNA. This small molecule is present whether the ILTat 1.3 gene is expressed or not. It may be some form of intermediary in the normal nuclear gene rearrangement associated with antigenic variation.

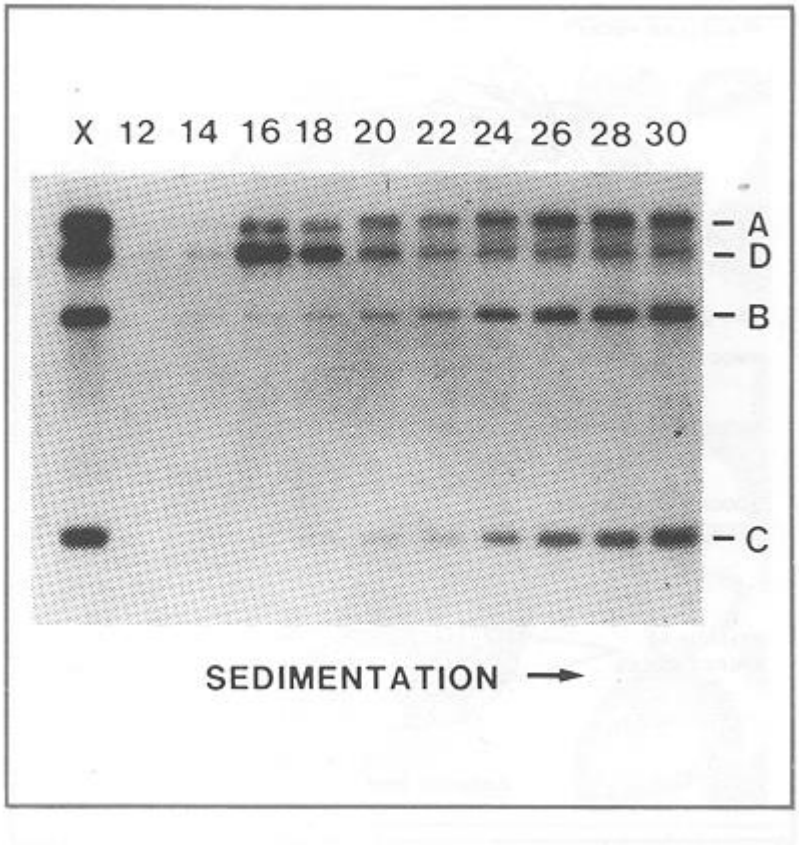

Figure 17. *Location of ILTat 1.3 genes in fractions from intact trypanosome DNA sedimented through a sucrose density gradient. Trypanosomes homogeneously expressing different VSGs were isolated by Percoll density gradient centrifugation. The trypanosomes were lysed by adding SDS to 0.5%. The lysate was incubated for 2 h with 100 mg ml^{-1} RNase A (previously heated to 70°C for 60 min), then for 4 h with 1 ml^{-1} of Pronase (preincubated at 37°C for 2 h), both at 37°C. The extremely viscous DNA solution was layered onto a 10–40% sucrose gradient in 10 mM HCI, 5mM EDTA pH 8.0 and centrifuged at 24 000 rpm for 10 h. The gradient was fractioned with an ISCO gradient fractionator (30 × 1.2 ml fractions). The position of the ILTat 1.3 genes in the gradient was determined by a Southern blot analysis of DNA from alternate fractions digested with Hincll, using pcBC1 as a probe. Tracks marked × are digests of*

unfractionated DNA. A, D, B and C mark the Hincil fragments from the four distinct copies of the ILTat 1.3 gene. Copy D is purified at fraction 16, while the other copies are purified at fraction 30. This shows that copy D is smaller and can be separated from the others.

Antigenic variation: synthesis and maintenance of the surface antigens

The surface antigen coat is probably the only component found in common on all African trypanosomes. The integrity of this structure is crucially important to the survival of the parasites in the host bloodstream. For this reason, biochemists at ILRAD are investigating how the VSGs are synthesized in the parasite and exported to the cell surface. A thorough knowledge of this process may suggest some form of interference which will disrupt the stability of the surface coat and render the trypanosome susceptible to host defence mechanisms.

Previous work at ILRAD and elsewhere has shown that *T b brucei* and *T congolense* VSGs contain two different types of carbohydrate (CHO) side chains, added to the protein by a process called glycosylation. The first is constructed in a region of the parasite cell called the endoplasmic reticulum (ER) shown in the simplified diagram of a trypanosome (Figure 18). It is attached to the protein by a linkage found in mammalian and other systems, and its addition can be blocked by the antibiotic tunicamycin. The other CHO side chain is linked near the end (C-terminal) of the protein by a mechanism which has not yet been described in any system and which cannot be blocked by tunicamycin. Its importance is suggested by evidence that it remains constant for all VSGs of *T b brucei* and *T congolense.*

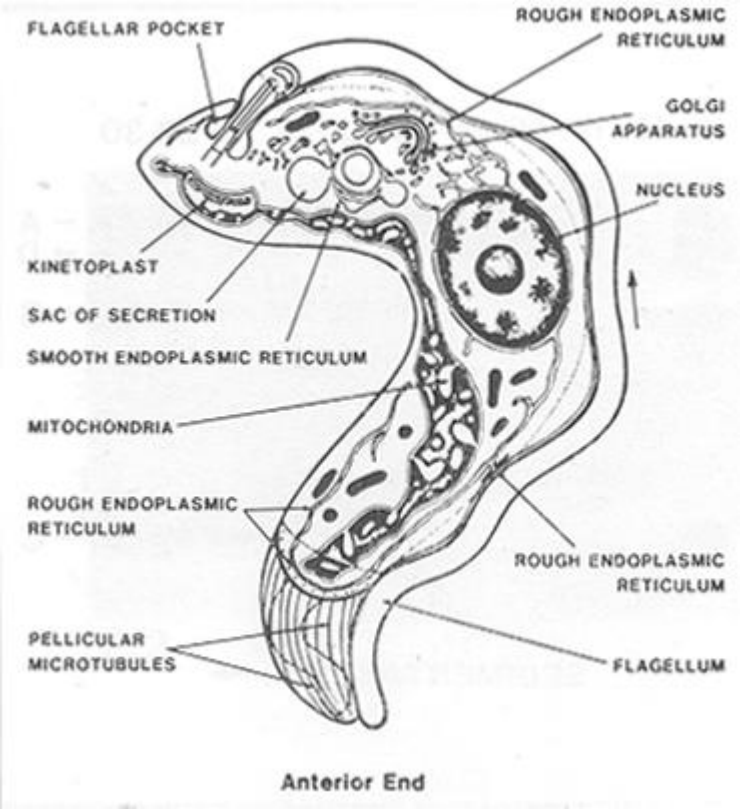

Figure 18. *Bloodstream form of* T congolense. *Derived from K Vickerman, 1969,* Journal of Protozoology, *16: 56.*

Figure 19. *Electron micrograph of* T vivax *rough endoplasmic reticulum (a), smooth endopiasmic reticulum (b) and Golgi (c). Endoplasmic reticulum and Golgi fractions were isolated from total microsomes obtained from* T b brucei, T congolense *and* T vivax *and tested for glycosyltransferase activities. Trypanosomes were disrupted in 250 mM sucrose-HKMM (50 mM HEPES, 25 mM KCI, 5 mM* $MgSO_4$*, 10 M MnC12; pH 7.4 at 5°C). Crude microsomes were prepared and a Golgi fraction isolated essentially after the method of Redman et al, 1975 (*Journal of Cell Biology, 66: 42*). Both smooth and rough endoplasmic reticulum fractions were prepared from the residual microsomal fractions according to the method of Ragland et al,* 1971 *(*Biochemical Journal, 121: 271).

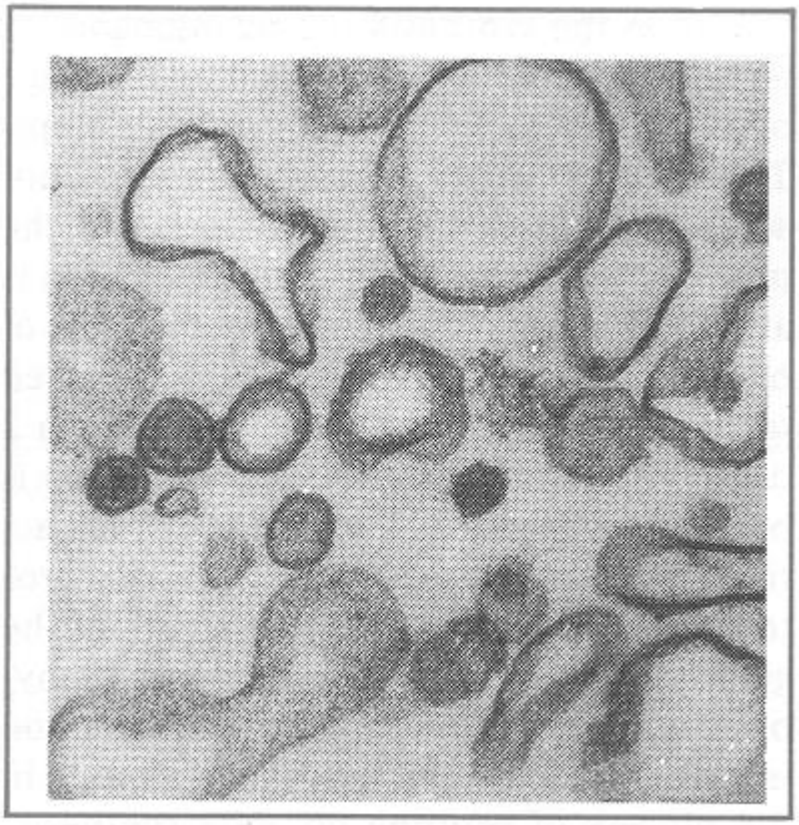

Figure 19(b).

It is more difficult to purify VSG from *T vivax* than from *T b brucei* or *T congolense* using available methods. However, ILRAD scientists have recently purified a VSG-like protein from a *T vivax* clone and are now conducting studies to determine how this molecule(s) compares biochemically and immunologically with VSG isolated from the other trypanosome species.

The synthesis of trypanosome VSG involves three subcellular organelles: the protein molecules are first synthesized on the rough ER and are then transported by the smooth ER to the Golgi apparatus. The CHO side chains are added during this process. The function of the side chains is unknown, but they may play a role in bringing the glycoprotein to the membrane surface. They may also enable the Golgi apparatus to recognize the VSG. This is important because the Golgi processes and sends glycoproteins to a variety of other cellular locations.

In 1982, ILRAD biochemists purified fractions of Golgi and rough and smooth ER in order to identify which of these subcellular organelles is critical to the synthesis of the VSG. This work was carried out in collaboration with Harvard University.

Golgi and ER fractions were isolated from *T b brucei T congolense* and *T vivax* and checked for morphological purity by electron microscope examination. Figure 19(a) is an electron micrograph of a rough ER fraction from *T vivax,* showing spherical and flattened vesicles with

irregularly arranged attached ribosomes. Clusters of free ribosomes, fragmented microtubules and other non-membrane material are also present. As shown in Figure 19(b), the smooth ER fractions from *T vivax* consist primarily of clear granular vesicles 30–200 nm in diameter. A few vesicles contain some flocculent material, but vesicles with attached granules are rare. All T vivax Golgi fractions contain flattened u-shaped, curved or cup-shaped cisternae with slightly expanded rims, as shown in Figure 19(c).

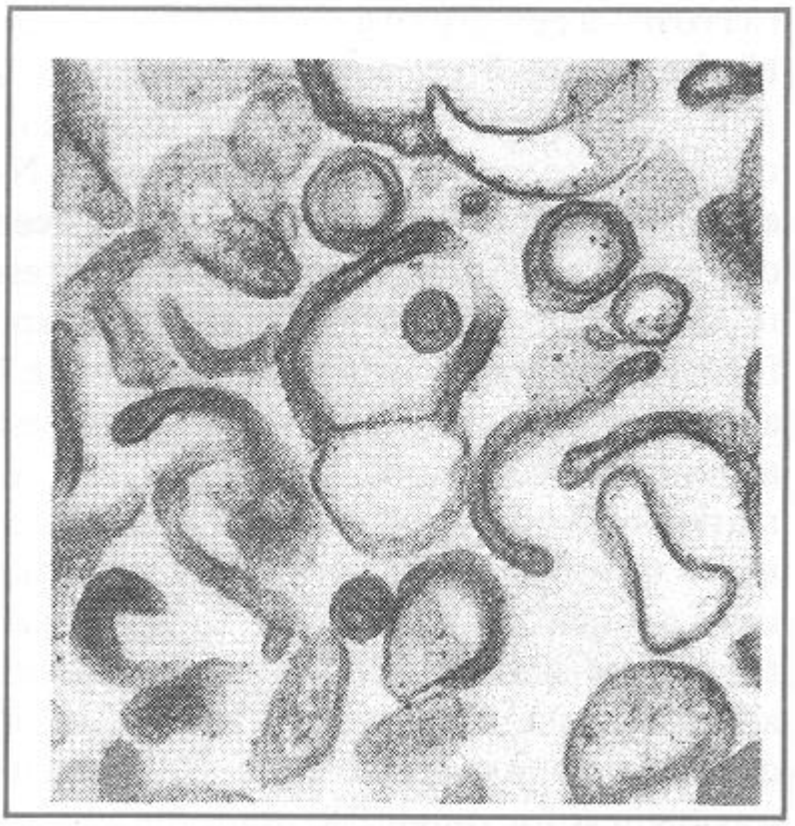

Figure 19(c).

The different glycosyltransferase enzymes involved in the process of glycosylation are being characterized biochemically. From total preparations of *T b brucei,* galactosyltransferase, mannosyltransferase and two different N-acetylglucosaminyltransferases have been characterized. Studies are now in progress to clarify the possible role of sialyl- and fucosyl-transferases using the purified ER and Golgi fractions. The glycosyltransferase activities observed so far have been remarkably similar in all fractions of the three major trypanosome species. The Golgi fractions contain the majority of the galactosyltransferase activity, followed by the smooth and then the rough ER. The dolicholdependent mannosyltransferase activity was highest for rough ER, then for smooth ER and then for Golgi.

The dolichol-independent N-acetylglucosaminyltransferase activity was similar in all the fractions tested, while the dolichol-dependent N-acetylgluco-saminyl- transferase activity was

much higher for ER than for Golgi, and somewhat higher for smooth than for rough ER. This enzyme is sensitive to tunicamycin inhibition in smooth ER, but not in rough ER or in Golgi, which suggests that it may take two or more forms. It also suggests that the core carbohydrate may be added to the VSG in the smooth ER. Experiments are currently in progress to see if these membrane fractions can process a mature VSG in vitro.

Another perspective on the factors involved in the synthesis and maintenance of trypanosome VSGs is provided by the stage of development when VSG synthesis stops. This occurs when bloodstream trypanosomes change into uncoated forms in the midgut of the tsetse fly. A culture system is available in which an entire population of bloodstream trypanosomes can be induced to transform into the fly midgut stage in 2 days. The termination of VSG synthesis is being investigated by incorporating radioactive amino acids into newly synthesized protein and looking for rearrangements of the genes encoding for VSG by gene probe hybridization to Southern blots. This method is being used to analyse trypanosomes in the process of shutting off VSG synthesis, as well as trypanosomes many generations after VSG synthesis has ceased.

Cultivation of trypanosomes in vitro

Cell biologists at ILRAD have made steady progress in the development of techniques to propagate the various stages of *T b brucei, T congolense* and *T vivax* in vitro. In collaboration with other scientists, these techniques have been used to study the biological, biochemical and immunological properties of the parasites. A great deal of trypanosomiasis research is now possible which could not be carried out in the past when trypanosomes could only be maintained by continuous passage through tsetse vectors and mammalian hosts.

Beginning with *T b brucei*, techniques have been developed to propagate and maintain trypanosomes in cell culture throughout all the stages of their life cycle (see Figure 20). Recent work has focused on extending these techniques to the cultivation of *T congolense* and *T vivax.* Once a system for maintaining trypanosomes is proven successful on an experimental basis, it can be standardized and used to produce large numbers of parasites which are required for many different research projects.

	T b brucei	T congolense	T vivax
Bloodstream forms (coated trypomastigotes)	yes	up to 35 days	up to 7 days
Procyclic forms (uncoated trypomastigotes)	yes	yes	yes
Epimastigotes	Yes	yes	yes
Metacyclics	Yes	yes	no
Complete lifecycle on continuous basis	Yes	no	no

Figure 20. *Stages of trypanosome development which can now be maintained in vitro.*

Using the culture system established for *T b brucei*, two simple in vitro tests were developed in 1982 to screen the effectiveness of trypanocidal compounds. The trypanocidal activity of a compound can be expressed in terms of the minimum drug concentration required to inhibit trypanosome growth by significantly more than 50% during 24 hours' exposure. Nine compounds were screened in 1982, with results shown in Figure 21. The tests being developed also measure any toxic side-effects on the bovine feeder cells included in the culture system. These two drug-screening procedures are now being applied to *T b brucei* field isolates. Tests are also being developed to screen the effectiveness of trypanocidal drugs against *T congolense* and *T vivax*. This work is carried out in collaboration with the Kenya Trypanosomiasis Research Institute and the Gesellschaft für Technische Zusammenarbeit (GTZ) of the Federal Republic of Germany.

Compound (trade name)	Supplier	MIC_{50} (g/ml)
Diminazene aceturate (Berenil)	Farbwerke Hoechst	1.00
Isometamidium (Samorin)	May 8 Baker	10.00
Phenanthridinium bromide (Novidium)	Boots	3.00
Quinapyramine dimethylsulfate (Antrycide)	Imperial Chemical	0.10
Melarsporol B.P. (Arsobal)	La Specia	0.01
Suramin (Naganol)	Bayer	1.00
α-difluoromethylornithine	Merrell-Dow	300.00
4', 6-diamidino-2-phenylindole	Serva	1.00
Quinapyramine isethionate	May & Baker	1.00

Figure 21. *Minimum inhibitory drug concentration (MIC_{50}) required to inhibit in vitro growth of* T b brucei *bloodstream forms by significantly more than 50% during 24 h exposure.*

The method of propagating *T congolense* metacyclics in vitro has been simplified and improved. Cultures are now initiated with bloodstream forms taken from infected rodents and raised in the presence of purified bovine dermal collagen. Six populations of *T congolense* parasites are maintained on a continuous basis, four from clones and two derived from uncloned stocks.

Work with *T vivax* has concentrated on two stages of parasite development: the surface-coated trypomastigote which develops in the bloodstream and the uncoated trypomastigote which is presumably equivalent to the next stage of development after the trypanosome is ingested by a tsetse fly. A culture system was tested in 1982 which maintains the coated bloodstream form for several days and a new system was developed in which coated parasites are induced to change to the uncoated form.

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Confirming results of previous research, bloodstream forms of a West African *T vivax* stock (Zaria Y 486 derivative) were isolated from a goat and maintained in vitro in collaboration with

the Swiss Tropical Institute. The parasites multiplied in culture and showed the *T vivax* bloodstream-form morphology. Best results were obtained when Microtus montanus (mountain vole) whole embryo fibroblasts were used as the feeder layer. A similar effort to propagate two East African *T vivax* stocks (ILRAD 1875 and KETRI 2377) in culture has not yet been successful.

Bloodstream trypomastigotes from the West African *T vivax* stock can now be taken from infected mice, maintained in culture and induced to change to the uncoated form. After being kept at 25°C for 3 days, the parasites are no longer infective to mice; examination with the electron microscope reveals that they lack a surface coat. Using this method, 10^9 or more of the uncoated forms can be produced at a time.

Several interesting findings have resulted from this work. For one thing, scientists at ILRAD have now observed rapidly dividing short-slender forms of *T vivax* with the kinetoplast at the posterior end during periods when parasite populations are rising (see Figure 22) and non-dividing elongated-slender forms with the kinetoplast midway between the posterior end and the nucleus during periods of peak parasitaemia (see Figure 23). Short tadpole-like forms have also been observed which are possibly senescent. These can also be seen in Figure 23. This pleomorphism is more pronounced when *T vivax* is raised in mouse blood than in goat or cattle blood. In vitro experiments have shown that the short-slender forms are more easily maintained in culture than the elongated-slender forms. However, the elongated forms change more readily in culture into the uncoated stage.

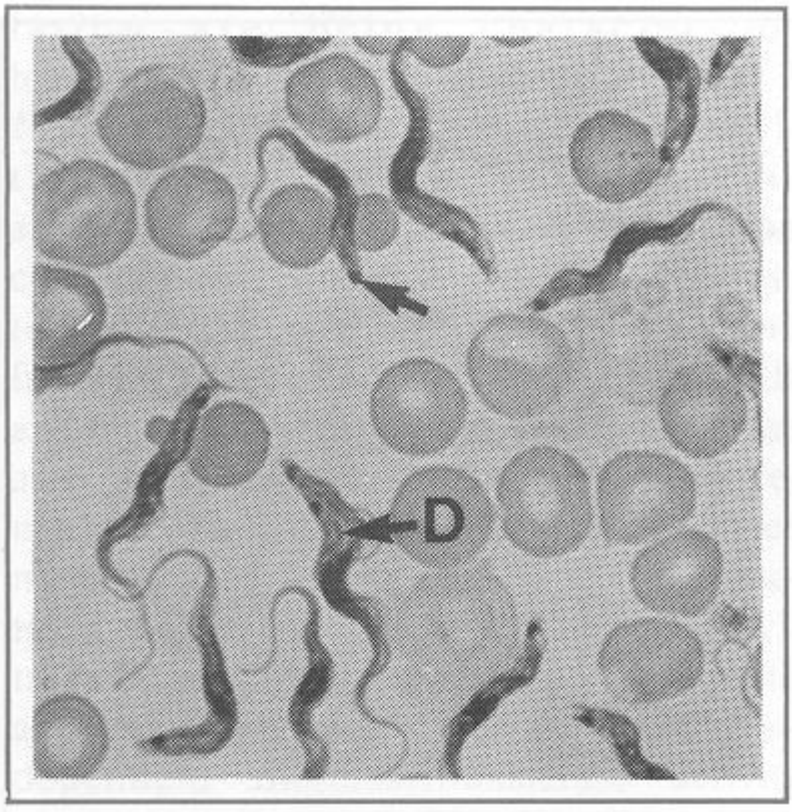

Figure 22. *Bloodstream forms of* T vivax *(ILRAD 1392) during rising parasitaemia in a mouse. There are many dividing forms (D). Note the position of the kinetoplast, indicated by the arrows, at the posterior end*

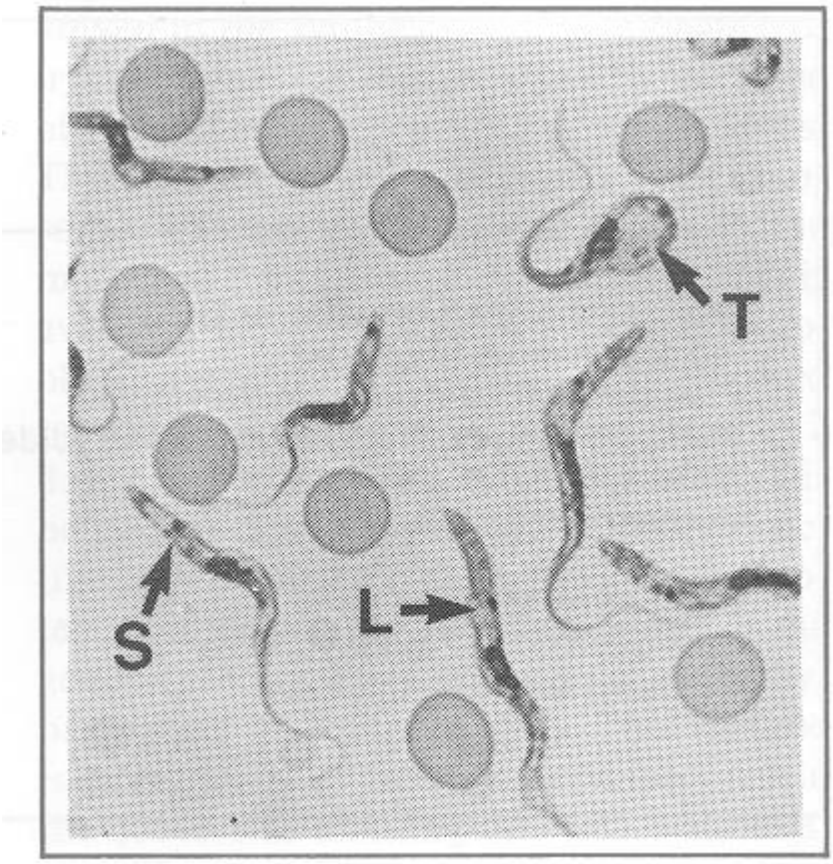

Figure 23. *Bloodstream forms of* T vivax *(ILRAD 1392) in a mouse at the peak of parasitaemia. Note the pleomorphism: some parasites are elongated (L) with the kinetoplast midway between the nucleus and the posterior end, while others are shorter (S), with the kinetoplast at the posterior end, or tadpole-shaped (T).*

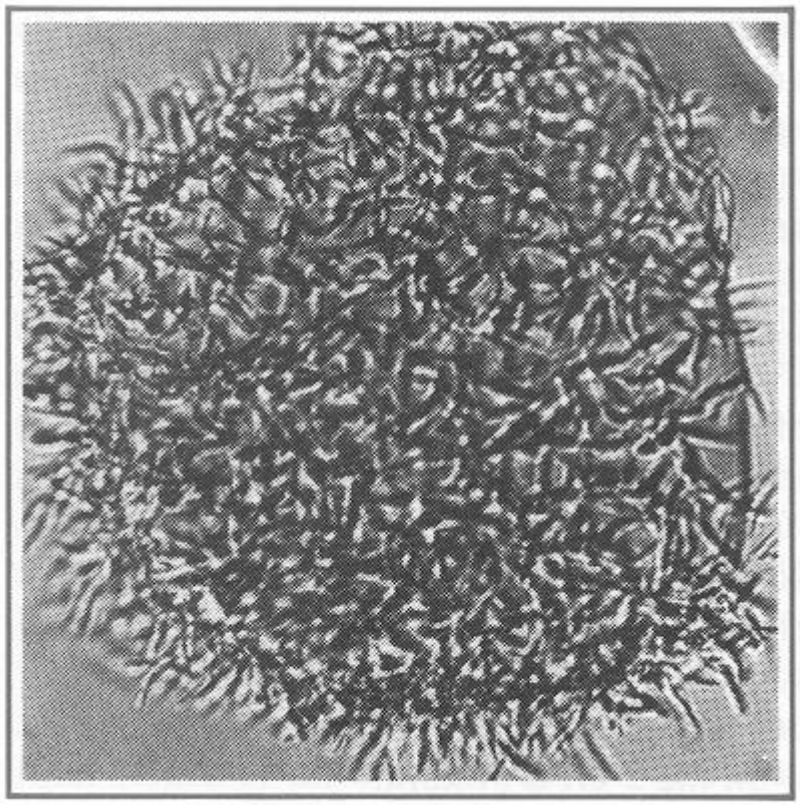

Figure 24. *Uncoated forms of* T vivax *(ILRAD 1392) transformed from the bloodstream form at 25°C in vitro. Under these conditions, they attach to the surface of matrix Green Gel A beads (Amicon) within 3 days. These parasites are noninfective to mammalian hosts.*

When uncoated *T vivax* trypomastigotes were cultured with Matrex Green Gel A beads (Amicon), many of them became attached to the beads at their anterior end, as shown in Figure 24. These parasites probably attach themselves in a similar way to the mouth parts of tsetse flies. Uncoated forms of other trypanosome species do not become attached to the gel beads in culture, so this difference in behaviour might be used to identify *T vivax* in vitro. When uncoated *T vivax* trypomastigotes transform further to the shorter epimastigote forms, those parasites which were attached to beads survive longer than those which were not. The *T vivax* culture system is now being developed further in an effort to support transformation to the metacyclic stage and back to the bloodstream form, thus completing the parasite lifecycle.

Work was initiated in 1982 on the biochemical characterization of forms of *T vivax, T congolense* and *T b brucei* propagated *in vitro*. This work has been undertaken in collaboration with a visiting scientist from the University of Ibadan in Nigeria.

Detailed studies of the parasite lifecycle

Metacyclic forms of *T b brucei* are injected in the bite of an infected tsetse fly, and change to long-slender forms which multiply rapidly in the mammalian bloodstream. Many of the long-

slender parasites differentiate into intermediate and then short-stumpy forms which do not multiply and eventually die out. The parasite population usually rises and falls in a series of waves associated with this rapid multiplication and differentiation to the senescent form.

It has been postulated that a similar differentiation from a rapidly dividing to a non-dividing bloodstream form occurs in *T congolense* and *T vivax*, though the physical changes are less obvious. Significant evidence was obtained in 1982 that such a differentiation does occur in these two trypanosome species, indicating that this is an important phenomenon which might present a target for disease control.

Different stages in the parasite lifecycle are characterized by different DNA contents. Non-dividing cells contain a baseline amount of DNA, while in an actively dividing population many cells have double the baseline DNA content in preparation for cell division. Using ILRAD's fluorescence-activated cell sorter, two actively dividing forms of *T b brucei*, procyclic forms equivalent to those which develop in the tsetse midgut and long-slender bloodstream forms, were shown to contain more DNA than non-dividing metacyclic and short-stumpy bloodstream forms. An analysis of early and late *T vivax* bloodstream populations revealed a similar transition from a dividing state to a non-dividing state with less DNA. This analysis is now being extended to *T congolense.*

Other studies focus on the proteins which are specific to different stages of the *T b brucei* lifecycle. The aim is to identify proteins which could serve as targets for immunological or chemotherapeutic attack. In 1982, proteins were examined which are specific to the long-slender and short-stumpy bloodstream forms and to the uncoated procyclic form. The protein composition of whole parasites at these different stages was analysed using two-dimensional gel electrophoresis. Several differences appeared, but the background of thousands of unchanging proteins made it difficult to detect which changes were significant. For this reason, the nuclei of trypanosomes in the three stages are being purified and analysed using the same procedure.

Many physiological processes similar to trypanosome differentiation are mediated through the action of a limited set of structurally related regulatory proteins which depend on calcium. Recently, ILRAD scientists detected one of these, calmodulin (CaM), in *T vivax.* This protein is now being purified and analysed biochemically. Preliminary evidence suggests that it is smaller than vertebrate CaM. Scientists elsewhere have found that CaM isolated from *T b brucei* is also smaller than vertebrate CaM. In addition, CaM-like activities in *T b brucei, T congolense* and *T vivax* do not cross react well with mammalian CaM when tested by radioimmunoassay. These are important findings because if CaM in fact plays a role in the differentiation process and if trypanosome CaM differs biochemically from the mammalian protein, then it may be possible to treat animals to inhibit this molecule and disrupt the parasite lifecycle without interfering with the host.

Trypanosome respiration and energy production systems have also been shown to change at different stages of the parasite lifecycle. Procyclic trypanosomes possess a fully functional mitochondrion and their respiration depends in part on the conventional cylochrome electron transport system. Bloodstream forms, however, appear to lack cytochromes; their energy is produced by glycolysis using an L-glycerophosphate oxidase (GPO) transport system. Studies in

vitro have shown that respiration depends entirely on the -GPO system in bloodstream forms of *T b brucei*, *T congolense* and *T vivax*. The respiration of *T b brucei* procyclics raised in vitro depends in part (35%) on the cytochrome electron transport system and in part (65%) on the -GPO system.. For *T congolense* procyclics, 25% of respiration depends on the cytochrome system and 75% on the -GPO system, while respiration of *T vivax* procyclics raised in vitro for 4 days depends entirely on the -GPO system.

Towards Improved Host Responses

Several studies at ILRAD focus on the complex interactions between trypanosomes and their hosts. Research concentrates on factors which affect host antibody production and on other host responses which play a role in resistance to the parasites. A better understanding of these interactions could lead to several possibilities for improved disease control.

Immune Responses

When mice are infected with *T b brucei*, they produce specific antibodies directed against parasite VSGs, as well as a variety of unrelated antibodies. These antibodies are produced by one type of lymphoid cell, the B-cell. B-cells are stimulated to multiply soon after infection. This occurs well before antibodies are produced, particularly in susceptible mouse strains.

In 1982, five mouse strains were infected with a *T vivax* stabilate derived from West Africa. All mice of the most susceptible strain died within 10 days of infection, while none of the most resistant strain died within 10 days and 50% were still alive after 40 days. Specific immune responses were analysed using the immune lysis test. The most susceptible mouse strain produced no antibodies either specific to the parasite or non-specific, while the most resistant strain produced both specific and non-specific antibodies. Further experimentation showed that the susceptible mice were in fact capable of responding to parasite antigens. Spleen cells taken from the susceptible mice were analysed using the fluorescence-activated cell sorter, and their B cells were found to be dividing. Thus these cells, while stimulated to divide, are apparently inhibited from completing the final stages of differentiation into antibody secreting cells.

Studies in livestock have shown that immune responses to metacyclic trypanosomes are highly specific for particular parasite populations. When cattle or goats are infected by tsetse with metacyclic forms of *T congolense* or *T b brucei* and treated at least 15 days later with trypanocidal drugs, they resist rechallenge with the same trypanosome stock but are fully susceptible to challenge with different stocks of the same species. In 1982, further experiments were carried out to determine whether cattle would develop a similar resistance based on infection by intradermal inoculation, using metacyclic trypanosomes propagated in vitro. Cattle were infected in this way with metacyclic forms of *T congolense.* They were treated 60 days later with the trypanocidal drug diminazene aceturate (Berenil-Farbwerke Hoechst AG). Ninety days after treatment, they were challenged by inoculation with the same *T congolense* stock and were immune to reinfection.

Research was also carried out in 1982 to isolate and characterize the specific parasite VATs responsible for recurrent peaks of host antibody production. Three steers were infected with a *T*

congolense stock (ILNat 3.1) and two steers with a stock of *T b brucei* (MITat 1.2). Blood samples were taken every day and examined for the presence of infecting VATs using the IFA test. A reappearing *T b brucei* VAT has been identified and is now being cloned for further study.

When animals infected with trypanosomes are presented with unrelated antigens, they produce lower levels of specific antibody than uninfected animals, or none at all. In many cases, the capacity of infected animals to respond to trypanosome VSGs is also reduced. This depression of the immune response is more marked in infected animals which are susceptible to trypanosomiasis than in infected animals which are more resistant. Experiments in 1982 showed that spleen cells taken from mice infected with *T vivax* or *T b brucei* produce considerably more prostaglandin hormones than spleen cells of uninfected mice. The concentration of prostaglandins produced by spleen cells taken from infected mice reaches levels which other investigators have shown are sufficient to reduce the immune response in living animals. Among infected mice, spleen cells from more susceptible strains often produce more prostaglandins than spleen cells from resistant strains. The addition to the spleen cell cultures of trypanosomes which were coated with antibodies or killed by freezing and thawing dramatically increases the amount of prostaglandins produced, especially when the spleen cells are from infected mice. These findings suggest that prostaglandin synthesis in animals infected with trypanosomes may contribute to the depression of the immune response.

The results of this study also suggest that dead or antibody-coated trypanosomes provide the fatty acid precursor for prostaglandin synthesis or provide an enzyme, such as phospholipase A2, which makes fatty acids available. If this is the case, the degree of immunodepression in an infected animal may be related to the number of parasites which multiply and die in the bloodstream. Further work concentrates on identifying the type of spleen cell which produces prostaglandins and the role of these cells in regulating parasitaemia and the immune response.

Other host responses

Host capacity to control parasite growth rates appears to be a key factor in resistance to trypanosomiasis. Scientists at ILRAD are studying host reactions which may limit trypanosome growth and development in the skin at the site of an infected tsetse bite, in the lymphatic system, and in the bloodstream. Studies also cover responses in the central nervous system at later stages of infection.

The local skin reaction

The chancre which develops in the skin at the site of an infected tsetse bite represents the host's first response to the parasite. When resistant wild animals are bitten by infected tsetse flies, they develop much smaller chancres than susceptible livestock. The prepatent period is also much longer before parasites can be detected in the blood. This suggests that the resistance of wild species is related to a capacity to control the parasites in the skin before they reach the bloodstream. Investigations at ILRAD concentrate on the possible role of the local skin reaction in conferring resistance, in particular on the structural changes and the distribution of parasites in the chancre and on the specific parasite material which induces the skin response. This work may

indicate how parasite proliferation is controlled in the skin and may provide markers for genetic resistance.

In one study, cattle were challenged by tsetse flies infected with *T congolense.* Skin biopsies and blood were collected at regular intervals and anaemia and parasitaemia were monitored. The biochemical analysis of tissue taken from the chancres revealed a variety of proteins and peptides (see Figure 25). Some of these have been shown to be aminopeptidases, while others are as yet only partially characterized. It is not yet clear whether these chancre-associated proteins and peptides are of host or parasite origin. The next step will be to characterize chancre-associated proteins, peptides and other molecules from animals which are resistant to trypanosomiasis.

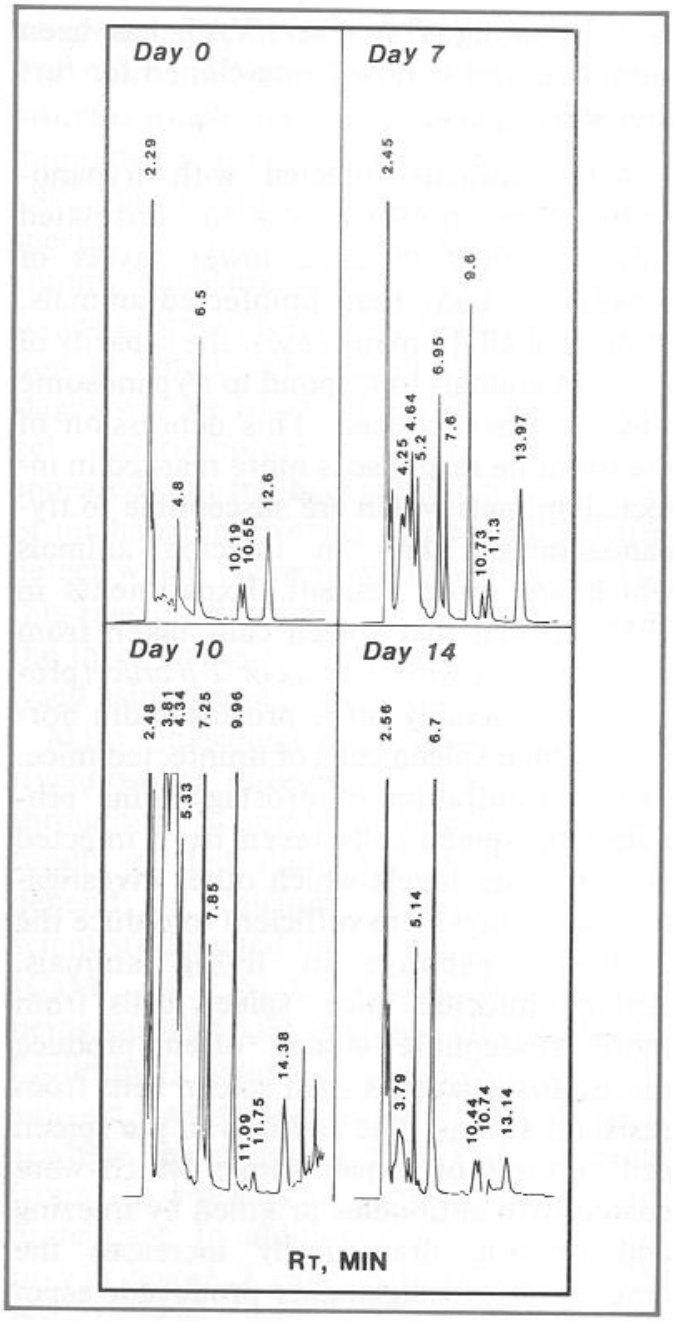

Figure 25. *Reverse phase high performance liquid chromatographic (HPLC) analysis of bovine chancre-associated peptides. This chromatogram shows the resolution of 80% ethanol-soluble peptides and other small molecules obtained from chancre extracts taken on days 0, 7, 10 and 14 after cattle were challenged by tsetse infected with* T congolense. *The study reveals a progressive appearance of molecules with retention times (Rt) between 3.8 and 5.33 minutes, reaching a 50-fold increase on day 10. Two other major chancre-associated peptides with approximate R_ts of 7.0 and 9.6 minutes show increases of 14- and 6-fold respectively.*

In a related study carried out in collaboration with the Swiss Tropical Institute, goats and cattle were challenged by tsetse flies infected with the three major trypanosome species, and biopsies were taken of chancres at intervals after infection. Chancre formation in these animals will be compared with skin reactions produced in infected buffalo.

Goats were also inoculated intradermally with bloodstream forms of *T vivax*, T *b brucei* and *T congolense.* Using this method, it was possible to measure how many trypanosomes were injected in the skin and to rule out the effects of tsetse saliva. With *T vivax* and *T b brucei,* a skin reaction was produced which appeared identical to a chancre. The onset, size and duration of the skin reaction depended on the number of trypanosomes inoculated: as few as 10^2 *T b brucei* parasites were sufficient to produce a chancre, whereas 10^4 *T vivax* were required. With T *congolense*, no chancre developed even when 10^7 bloodstream parasites were inoculated. .

When an animal is bitten by an infected tsetse fly, uncoated procyclic trypanosomes may be injected into the skin along with the metacyclic forms. To test the possible role of procyclic trypanosomes in stimulating chancre formation, goats were inoculated intradermal with procyclic parasites. When 10^8 *T b brucei* or *T congolense* procyclics were inoculated, an intense inflammatory reaction was detectable during the first 3 to 4 days. Smaller numbers of parasites did not produce a reaction.

Another study is in progress to determine what parasite material stimulates the skin reaction. *T congolense* metacyclics have been collected from tsetse flies and placed in millipore diffusion chambers. Chambers with a pore size of 0.45. µm have been implanted under the skin of goats. A definite skin reaction occurs, similar to, but smaller than a chancre, despite the fact that the trypanosomes are unable to exit from the chamber and initiate infection.

The role of the lymphatic system

Trypanosome have been observed in lymphatic vessels in the region of the chancre and in enlarged lymph nodes draining the site of infected tsetse bites. In 1982, investigations continued on the role of the lymphatic system in the development of trypanosome infections.

Calves were challenged by tsetse infected with *T congolense* and the lymph nodes draining the resulting chancres were cannulated. Lymph flow was measured and the cellular and parasite content of the lymph was monitored. Trypanosomes were detected in efferent lymph 3 to 5 days before they were observed in the bloodstream, as shown in Figure 26. Antibodies specific to the infecting parasite clone were detected in both lymph and plasma 2 weeks after infection. Further work will concentrate on typing lymphatic cells in the chancre, the efferent lymph and the bloodstream and identifying which cell types respond to trypanosome antigen.

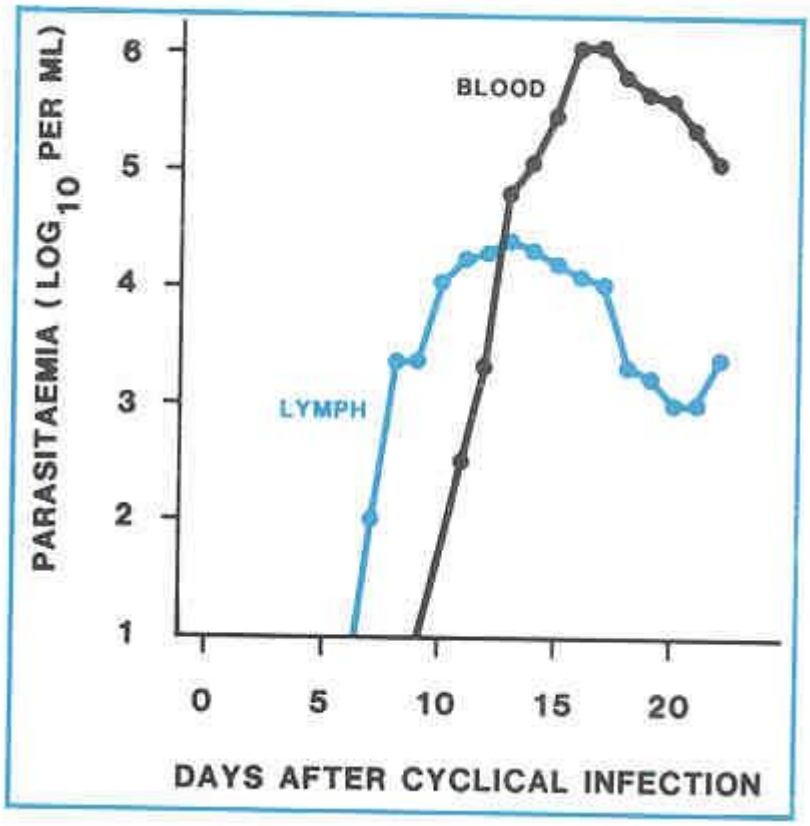

Figure 26. *Parasitaemia in the lymph and blood of calves infected with* T congolense. *Trypanosomes were always detected earlier in lymph than in blood, but the peak levels of parasitaemia were 1 to 2 logs higher in the bloodstream.*

Control of parasites in the bloodstream

For *T b brucei* and possibly for other trypanosome species, the level and duration of parasitaemic waves in the bloodstream appear to be related to parasite differentiation from an actively dividing to a non-dividing form. Earlier research has shown that some factor in the host plays a role in the timing of parasite differentiation and thus in the level of parasitaemia, because differentiation occurs at different times in different host species and strains, even when the parasite population is genetically identical.

The pattern of host resistance to *T b brucei* has been studied in detail in mouse strains which are relatively resistant or susceptible to trypanosomiasis. Trypanosome populations rise to a peak in the bloodstream as long-slender parasites divide. The level and duration of these parasitaemic peaks appears to depend on the timing of differentiation from rapidly dividing, long-slender parasites to the senescent short-stumpy forms. Studies in 1982 confirmed that trypanosome differentiation is slower in susceptible mouse strains. This slower differentiation is accompanied by a delayed host antibody response, but rapid parasite differentiation in resistant mice does not appear to be due to more efficient antibody production. Rather, host antibody responses seem

only to be effective against the parasites after a number of short stumpy forms have appeared and the expansion of the trypanosome population has already slowed down substantially.

Clearance studies with ^{75}Se-methionine labeled trypanosomes and adoptive transfer experiments have shown that antibodies produced against specific parasite VSGs do not destroy either long-slender or short-stumpy *T b brucei* parasites selectively, nor do they stimulate the parasites to differentiate to the short-stumpy form. Furthermore, parasite growth and differentiation rates were found to be the same in irradiated mice with or without reconstituted spleen cells, although antibody was detected in the spleen-cell-reconstituted mice and not in the others. This indicates that control of parasitaemia is not entirely dependent on the production of antibodies.

The development of *T b brucei* infections has also been compared in relatively susceptible 1-week-old calves and more resistant yearlings. As in mice, this study showed that resistance in cattle is apparently related to the ability to control parasite growth rates in the bloodstream and this in turn is related to the timing of parasite differentiation.

When mice were treated with *Corynebacterium parvum*, an adjuvant which enhances resistance to trypanosomiasis, and infected 4 days later with *T b brucei*, parasite differentiation occurred earlier than in controls. During the first 9 days after infection, peaks of parasitaemia were lower and of shorter duration in the treated mice, as illustrated in Figure 27. No difference was found in antibody production between the treated mice and the controls during the first 7 days after infection, which indicates that some other response was involved in the control of parasitaemia. The factors which regulated parasite growth and differentiation were partly radio-insensitive.

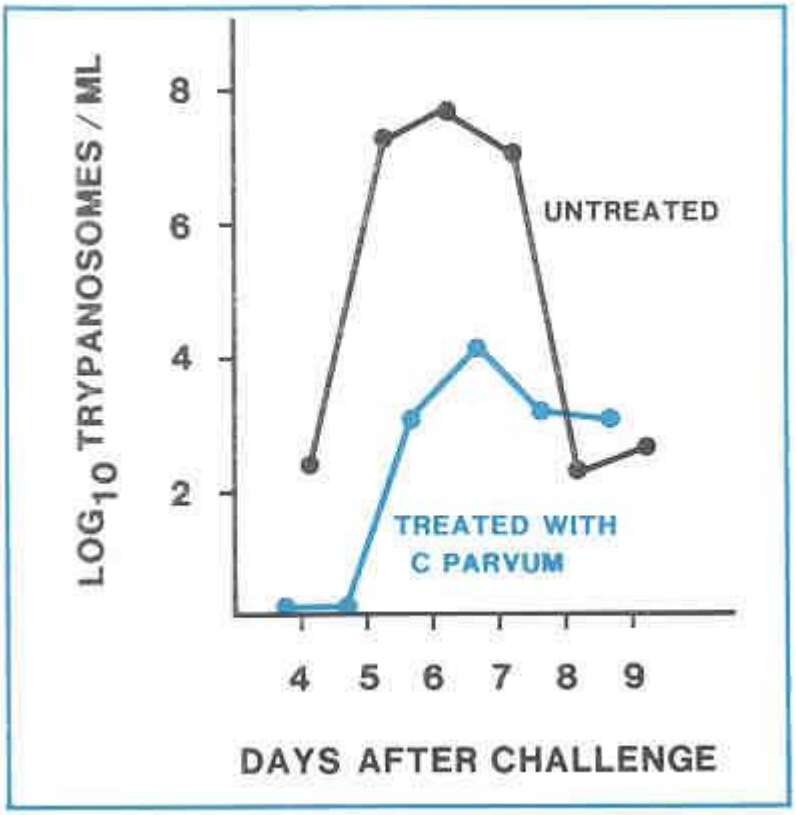

Figure 27. *When C57B1/6 mice were treated with* C parvum *4 days before challenge with* T b brucei*, the subsequent level of parasitaemia was significantly reduced.*

Resistance to trypanosomiasis, as measured by survival, appears to be an inherited trait involving several genes. Specific host responses which contribute to resistance may be under less complex genetic control. To investigate this possibility, the genetic bases of factors regulating the growth of *T vivax* and *T b brucei* in the bloodstream were compared in susceptible and resistant mice. Random matings were established between a susceptible and resistant strain, and offspring were tested for their capacity to control parasitaemia. F_1 crosses were then crossed with F_1s and bred back to both the parental strains. The results indicate that control of parasitaemia is based on a co-dominant gene system which is possibly fully as complex as the genetic basis of resistance measured in terms of survival.

To test the effects of host products on trypanosome survival, growth and differentiation rates, *T b brucei* parasites at different stages of development were maintained in a cell- and serum-free culture medium and exposed to a variety of substances they might encounter in the mammalian bloodstream. Without any additions to the medium, long-slender forms survived for 40 hours: during the first 5 hours they retained 100% infectivity for mice, from the 5th to the 20th hour they showed progressively less infectivity, and from the 20th to the 40th hour they developed into stumpy-like forms which were not infective for mice but were viable and had a surface coat. When moved to appropriate culture conditions, they differentiated to procyclic-like forms,

showing that they were not irreversibly committed to degeneration. Intermediate parasite forms survived for 20 hours in culture and underwent changes similar to those occurring in the long-slender forms in their last 20 hours of culture. Short-stumpy parasites survived in culture for 4 to 5 hours, undergoing changes similar to those occurring during the last 10 hours of culture of the long-slender and intermediate forms.

The addition of transferrin, bovine serum albumin, foetal bovine serum, normal mouse plasma or normal mouse serum to the cultures all extended the life-span of the parasites, but had no effect on declining infectivity. Thirteen other substances were tested, including prostaglandins, insulin and epidermal growth factor, but these had no effect on parasite viability or infectivity for mice. Spleen cells from normal or trypanosome-infected mice extended the parasite life-span, stimulated the parasites to multiply and slowed down their loss of infectivity. These effects were more pronounced when spleen cells were taken from relatively susceptible mouse strains than from resistant strains, while spleen cells from mice of intermediate susceptibility were intermediate in terms of their capacity to extend parasite survival and infectivity.

In a related study, actively dividing *T congolense* parasites were cultured in vitro with cells taken from resistant and susceptible strains of mice. Subpopulations of mouse spleen cells and peritoneal exudate cells were used. As in the previous study, the trypanosomes survived less well when cultured with cells from relatively resistant mice. Trypanosome viability was also lower when the parasites were cultured with cells taken from mice previously injected with *C parvum*, as shown for the peritoneal exudate population in Figure 28. When trypanosomes were cultured with cells taken from cattle or buffalo, the results also reflected relative susceptibilities in vivo: *T b brucei* parasites survived equally well when cultured with cattle or buffalo cells, but *T congolense* cultured with buffalo cells did not survive well. These systems are now being used for a detailed investigation of the mechanisms involved in host resistance to trypanosomiasis and may be useful in screening to identify relatively resistant animals.

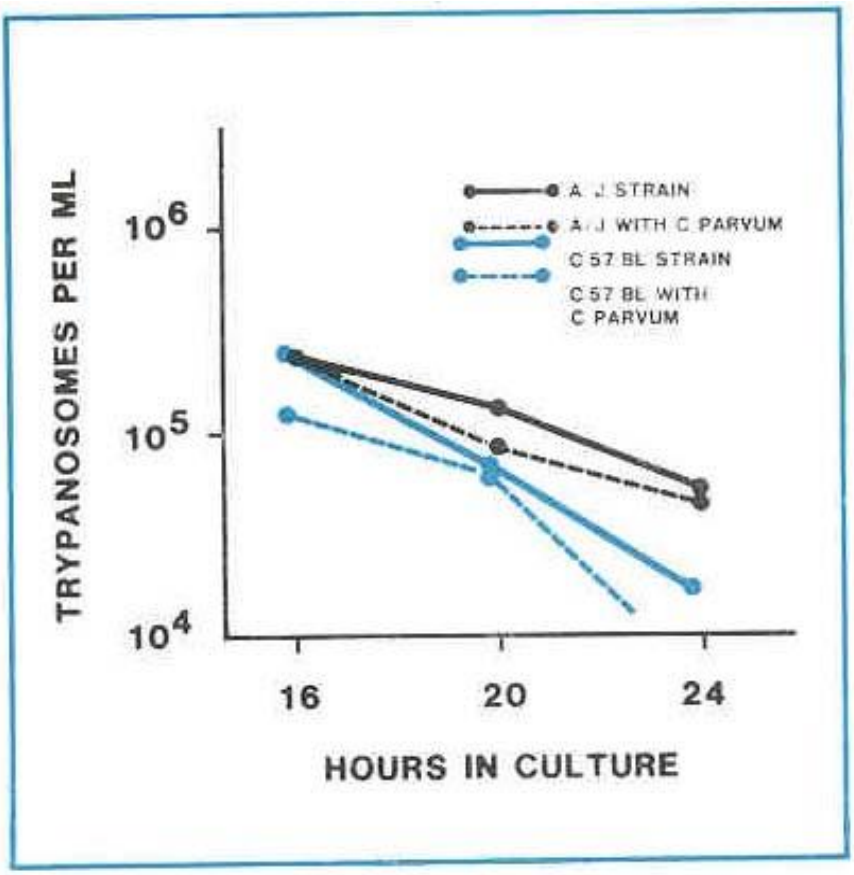

Figure 28. T congolense *cultured with peritoneal exudate adherent cells from two mouse strains: A/J mice which are relatively susceptible to the parasite in vivo and C57BI which are relatively resistant. The cells of the A/J mice maintained* T congolense *in culture much better than the C57BI cells. This difference became more marked when cells were used from mice previously infected with the immunostimulator* C parvum.

Effects of trypanosomiasis on the central nervous system

Cattle and goats infected with *T b brucei*, may develop a fatal illness characterized by clinical abnormalities in the central nervous system. This may occur during the initial infection or after treatment with trypanocidal drugs. Two projects were initiated in 1982 to investigate central nervous system involvement *in T b brucei* infecitions.

In one study, 12 cattle were infected with *T b brucei* or a mixture of *T b brucei* and *T congolense.* Sera and fluid were collected and analysed for the presence of antibodies specific to the trypanosome clones used. A slight rise in IgM antibodies was observed in the cerebrospinal fluid 2 months after infection.

The cattle given mixed infections developed abnormalities in the central nervous system and died. In 6 out of 8 of the cattle which died, a mild to moderate meningoencephalitis was apparent. Brain tissue from these animals is now being analysed for the presence of immune complexes.

In the second study, eight cattle were infected with *T b brucei.* Four of these animals died 4 to 6 months after infection. Despite mild anaemia and intermittent, low-level parasitaemia, they became very ill and emaciated and showed typical signs of central nervous system derangement as a result of severe meningo-encephalitis. The other four cattle were treated with the trypanocidal drug diminazene aceturate. They developed clinical signs after treatment and similar lesions were observed in their brains.

To investigate this syndrome further, 14 East African-Galla crossbred goats were infected with *T b brucei.* Unlike the cattle, they showed persistently high parasitaemia and developed severe anaemia. Three animals died 6 weeks after infection. The others were treated and all but one appeared to recover, but in the 3rd month after treatment half of the remaining goats developed anaemia with signs of central nervous system involvement, as shown in Figure 29. In all cases, trypanosomes were present in the cerebrospinal fluid, which also showed increases in protein and cell content. Brain tissue from these animals is being examined by electron microscopy to determine how the parasites invade the central nervous system.

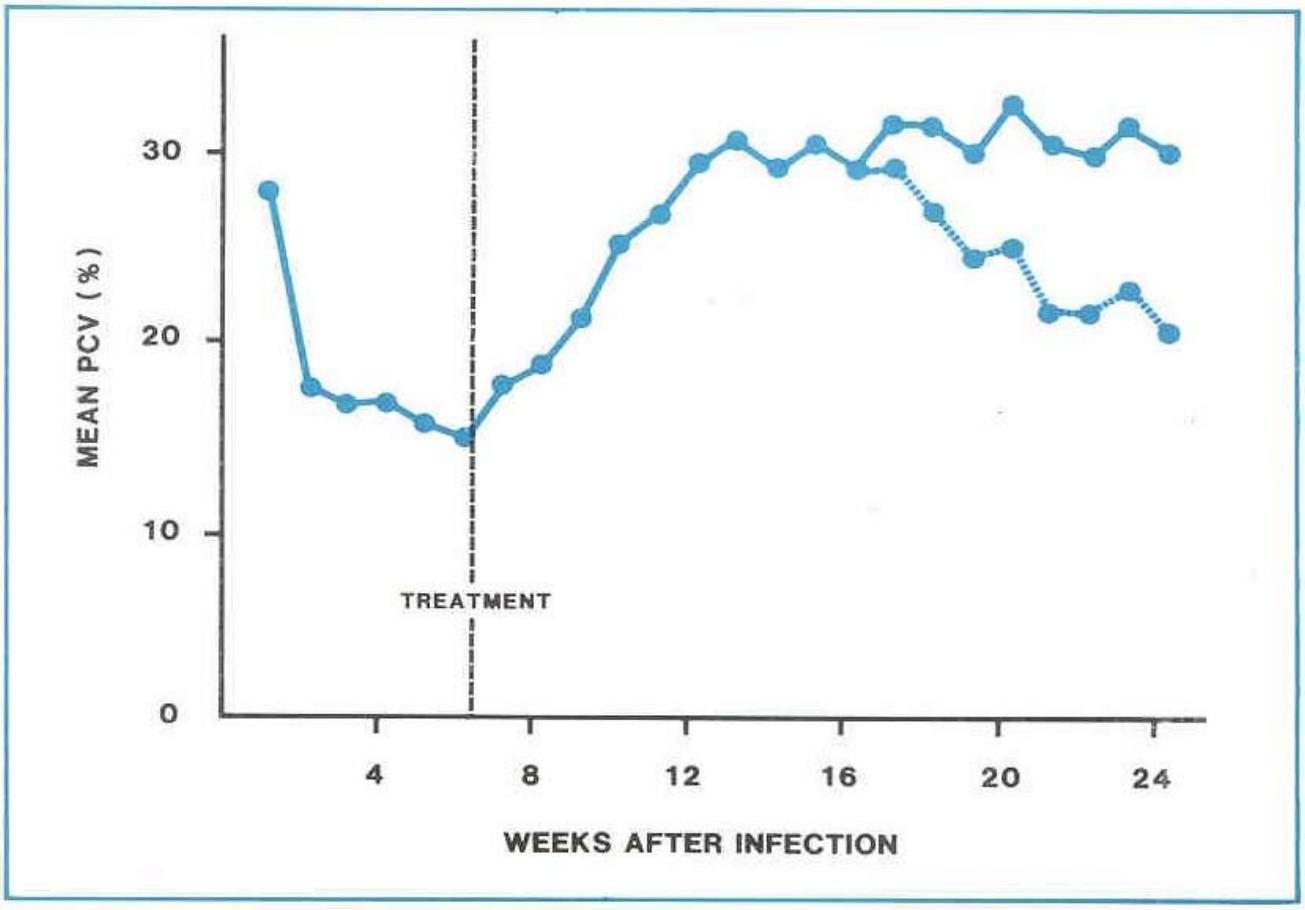

Figure 29. *Development of anaemia in goats infected with* T b brucei *and treated after 7 wks with diminazene aceturate. Fourteen goats were infected intravenously with* 10^6 T b brucei *(ILRAD 1797) and the course of anaemia measured by changes in packed cell volume (PCV). The figure shows mean PCVs up to 24 weeks after infection. Four goats died within 8 weeks, but*

after treatment the PCVs of the others returned rapidly to normal. However, beginning 9 weeks after treatment the infections in half the remaining goats relapsed. The average PCV of the relapsed group is shown by the broken line in the figure, while the continuation of the solid line shows the average PCV of the others.

Both studies confirm that *T b brucei* can be pathogenic for domestic livestock. In the second study, brain lesions were observed both after primary infections and after treatment with trypanocidal drugs. Parasites entering the central nervous system are resistant to the effects of many of the trypanocides commonly used to treat livestock because the drugs cannot cross the bloodbrain barrier. Thus relapsing infections which occur occasionally after treatment may not necessarily be the result of drug resistance or underdosage, but rather may be due to the transfer of parasites to the central nervous system and subsequent systemic reinfection.

Resistance to trypanosomiasis in livestock and wildlife

Comparative studies on host resistance to trypanosomiasis comprise an important research area at ILRAD. One goal is to find genetic markers which can be used to identify relatively resistant animals for livestock improvement programs. A better understanding of how some animals resist trypanosomiasis may also suggest promising interventions for increasing the resistance of others.

Previous work has suggested that indigenous East African livestock breeds may possess some genetic resistance to trypanosomiasis, though not to the same degree as the recognized West African trypanotolerant breeds. Studies are in progress to assess levels of resistance and determine the mechanisms involved.

In 1982, five groups of cattle of different breeds were infected with *T congolense* by intravenous injection. These were Boran and Ayrshire from tsetse-free areas of Kenya and three groups of Zebu from different tsetse-endemic zones. The Ayrshires were significantly more susceptible to trypanosomiasis than the other groups, followed by the Boran and the two Zebu groups from low tsetse-challenge areas, all with similar levels of susceptibility. The Zebu cattle from a high challenge area in western Kenya were generally most resistant, but this group showed considerable heterogeneity. Resistant animals controlled parasitaemia more effectively and developed less severe anaemia. The Ayrshire cattle produced less anti-trypanosome antibody than the other four groups. The other groups did not differ significantly in terms of antibody production, but antibody activity persisted longer in the more resistant animals. As the three Zebu groups had been exposed to trypanosomiasis previously, it was not possible to assess whether the resistance they demonstrated was due to innate or acquired factors.

In a related study, five groups of goats were challenged with *T congolense* by infected tsetse flies-East African goats, Galla and crossbreds of East African with Galla, Nubian and Toggenburg. No important differences were observed between these breed groups, either in the development of the chancre or in the timing, level or duration of the first parasitaemic wave. All groups developed severe anaemia by the 6th week after infection. Immune responses to the parasite, measured by immune lysis of bloodstream forms, were also similar for all groups.

Red Masai sheep from East Africa have been shown to resist trypanosomiasis better than imported Merinos. Parasite populations increase just as quickly in the Red Masai, but the resulting anaemia is milder. Studies are in progress to determine the reason for this relatively mild anaemia, but initial results have been inconclusive.

It has been observed for some time that wild animals resist trypanosome infection, but little is known about the mechanisms involved. ILRAD scientists have carried out several studies on resistance in East African wildlife species in collaboration with the Kenya Government's National Veterinary Laboratories, supported by the Government of the Netherlands.

To study the role of the local skin reaction and other responses to trypanosome infection, buffalo, oryx, waterbuck, eland, taurine cattle and East African goats were exposed to tsetse flies infected with *T congolense, T b brucei* or *T vivax*. Among animals exposed to T *congolense,* chancres appeared at 80 to 90% of infected bite sites in the domestic species and at 20 to 60% of bite sites in the wild species. The chancres which developed were also larger in the domestic animals. The prepatent period before parasites appeared in the bloodstream was generally much longer in the buffalo, oryx and waterbuck than in the goats and cattle, and the level of parasitaemia was lower in the wild species. All the animals showed some anaemia, as measured by reductions in packed cell volume **(**PCV), but only the domestic animals suffered from severe or chronic anaemia. These results, shown in Figure 30, suggest that the greater resistance to *T congolense* shown by the wild species may be related to better control of parasites in the skin and better control of anaemia.

	% Skin reaction at bite sites	% Skin thickness increase	Prepatent period (days)	Maximum parasitaemia (parasites/ml)	% Drop in PCV
Buffalo	20	0	32	10^{2}-10^{3}	5
Oryx	20	0	27	10^{4}	20
Waterbuck	60	70	23	10^{4}	24
Eland	40	100	13	10^{6}	24
Cattle	80	200	13	10^{6}	37
Goats	90	300	9	10^{6}	50

Figure 30. *Comparison of resistance to trypanosomiasis infection in wild and domestic animals, as shown by differences in local skin reaction, development of parasitaemia and level of anaemia. Animals were challenged by tsetse infected with* T congolense.

The pattern of reactions was similar among animals exposed to *T b brucei.* Eland and waterbuck developed fewer chancres and less severe anaemia, but the differences between the wild and domestic species were less marked than for *T congolense.* It was not possible to infect the eland or water buck with *T vivax* using tsetse flies, though the animals were fully susceptible to intravenous challenge with bloodstream parasites. Further experimentation will determine whether this resistance to tsetse challenge is due to responses in the skin or to a resistance to metacyclic, as opposed to bloodstream, forms of the parasite.

When eland, buffalo, waterbuck and cattle were infected intravenously with bloodstream forms of *T b brucei,* the first wave of parasitaemia was similar in all the animals. However, after the first wave, parasite levels were considerably lower in the eland and buffalo than in the cattle and decreased gradually until parasites could no longer be detected. In the waterbuck, several parasitaemic waves occurred, similar to those observed in the cattle. However, anaemia was severe in the cattle, but very mild in all three wild species. Clearly, some factor limits parasite growth in the bloodstream of eland and buffalo, perhaps responses similar to those detected in resistant mouse strains.

In another study, wildebeest and Boran cattle were infected intravenously with bloodstream forms of *T b brucei* and their antibody responses were compared. Both the wildebeest and the cattle produced IgM, $IgG_1$and IgG_2 antibodies directed specifically against trypanosome antigens, but the cattle produced much lower levels of IgM than the wildebeest. The wildebeest produced antibodies in a pattern of recurrent peaks, while the cattle did not. The cattle were able to control parasitaemia within 2 months after infection, whereas the wildebeest were still slightly parasitaemic after 7 months, but the cattle became anaemic and the wildebeest did not.

A culture system has been developed which should make it possible to study these reactions in more detail. Cell lines were established from embryonic tissue taken from eland, impala, Grant's gazelle and goats. These cells lines have been tested in combination with different sera for their capacity to support the growth of bloodstream forms of *T b brucei* and *T b rhodesiense.* Parasites were maintained successfully with feeder layers of eland, impala and goat cells combined with sera from the same species, but not with cells of Grant's gazelle. Further experiments are in progress to investigate why the parasites could not be maintained with the gazelle cells. The success of the other culture systems indicates that tissue and sera from eland and impala have no innate factors which inhibit parasite growth. These results support the observation that the mechanism which enables eland to control trypanosome growth in the bloodstream is only stimulated after an initial parasitaemic wave.

In vitro studies have demonstrated that the enzyme polyamine oxidase, present in the bloodstream of wild and domestic ruminants, suppresses the growth of African trypanosomes in the presence of either spermine or spermidine. High levels of polyamine oxidase activity have been detected in sera from adult buffalo, cattle, eland, sheep and waterbuck, while none was detected in non-ruminant sera. Much lower levels of activity were observed in fetal bovine sera than in sera from adult cattle, but activity increased rapidly in newborn calves, reaching adult levels on the 21st day after birth.

Spermidine and putrescine were detected in bloodstream forms of *T vivax* and *T b brucei* and in procyclic forms of all three major trypanosome species. No spermine was detected. When bloodstream and procyclic forms of *T b brucei* were cultured with bovine serum, the spermidine they released was broken down by polyamine oxidase, forming aldehyde which gradually decomposed to putrescine. The amount of spermidine in the culture increased for 42 hours and then dropped off: at the same time, the population of trypanosome bloodstream forms increased for 42 hours and then decreased slightly (see Figure 31). This suggests that the toxic aldehyde produced from the breakdown of spermidine may suppress the growth of trypanosomes in culture.

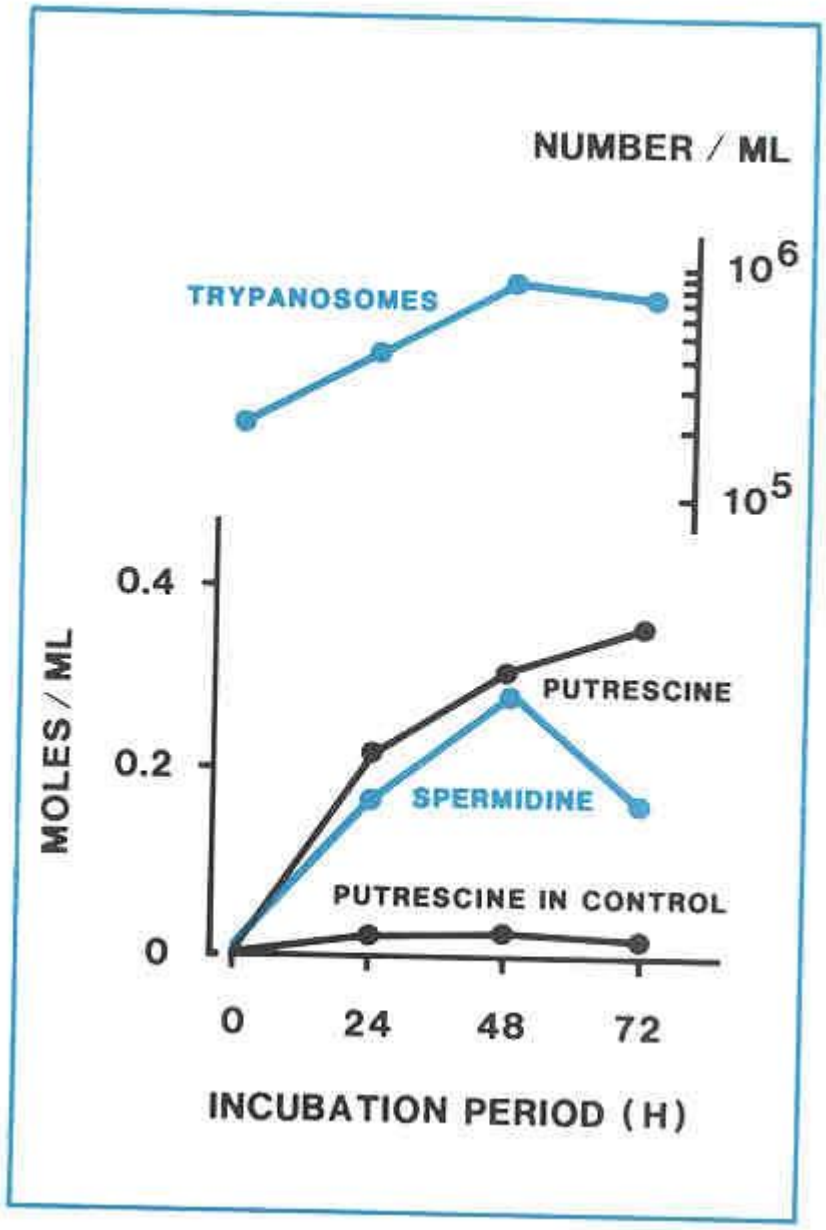

Figure 31. *Bloodstream forms of* T b brucei *were cultured in HEPES (25mM)-buffered RPMI 1640 medium with 20% fetal bovine serum at 28°C for 72 h. The number of trypanosomes reached a maximum concentration after 48 h and decreased slightly during the next 24 h. Likewise, the amount of spermidine released in the culture medium increased up to 48 h and decreased considerably during the next 24 h. The amount of putrescine, produced by the poiyamine oxidase activity of the serum, increased steadily throughout the 72 h period.*

Epidemiology

In many field situations in Africa, livestock are exposed to more than one trypanosome species and to a large number of genetically distinct parasite populations, called serodemes. The development of techniques to identify trypanosome species and serodemes is an important component of ILRAD's epidemiology program. The epidemiology program also includes studies on the role of tsetse flies as trypanosomiasis vectors. In addition, a field project is being conducted at the Kenya coast, and ILRAD scientists are participating in a collaborative research project on livestock productivity and trypanotolerance covering sites in several African countries.

Characterization of trypanosome populations

Scientists are developing techniques to identify trypanosome species based on the analysis of antibodies present in the blood of infected animals. Monoclonal antibodies have been raised against *T congolense* and *T b brucei* parasites and the antigens recognized by these antibodies have been isolated using immunoadsorbent columns. Antibodies against these purified antigens were readily detected by enzyme-linked immunosorbent assay (ELISA) in sera taken from cattle infected with the same trypanosome species, but not in sera from cattle infected with different species. Purified *T vivax* antigens are now being prepared using the same technique and antigens from all three species are being characterized biochemically. By detecting antibodies produced against these antigens, it is now possible to determine how quickly antibodies are produced after trypanosome infection and how long these antibodies persist in the bloodstream after an animal recovers. This approach could be extended to identify parasites in tsetse flies.

Within a trypanosome species, serodemes can also be identified by analyzing antibodies present in the blood of infected animals. Experiments using monoclonal antibodies and sera from infected rabbits indicate that metacyclic parasites of specific *T congolense* and *T b brucei* serodemes display a limited number of VSGs about 10 to 12 in the *T b brucei* serodemes studied and 4 to 6 in the *T congolense* serodemes. These metacyclic VSGs are characteristic and constant for each serodeme.

Cattle chronically infected with bloodstream forms of *T congolense* or *T b brucei* produce antibodies against metacyclic VSGs of the infecting serodeme, but not against metacyclic VSGs of other serodemes. This indicates that bloodstream forms produce VSGs which are the same or very similar to metacyclic VSGs of the same serodeme. This study is now being extended to *T vivax.* The long-term objective is to develop techniques which can be used to type trypanosome isolates and determine the number of serodemes present in any given field situation. The detection of specific antibodies against trypanosome metacyclics may also indicate whether animals under field challenge conditions have developed any immunity to the local serodemes.

Another system is being developed on an experimental basis to identify trypanosome species and serodemes by analysing parasite DNA. Two genes which code for specific VSGs have been cloned from two well characterized *T congolense* stocks. Double stranded DNA copies (cDNA) have been synthesized from these genes and cloned in bacteria. One of the cDNA clones is being

used to probe T *congolense* isolates from various regions of East Africa, primarily by Southern blot hybridization, to identify specific serodemes.

A third approach involves priming animals by infection with one serodeme and treatment, and then infecting them with various field isolates and monitoring the appearance of chancres. Goats develop chancres when they are primed with one serodeme of *T congolense* or *T b brucei* and then challenged with isolates of different serodemes, but no chancre develops when they are challenged with an isolate of the same serodeme as the original infection. This approach has not yet been successful in identifying *T vivax* serodemes.

It has also proven difficult to prime experimental animals with more than one serodeme. When goats were infected with four *T congolense* serodemes at 4- or 12-day intervals, the initial infection blocked subsequent infections so the animals developed full resistance to the first serodeme only. This interference may be due to a nonspecific response to the first infection which subsequently limits parasite growth in the skin. Another finding was that goats or cattle which were bitten by tsetse flies three or more times developed striking immediate hypersensitivity reactions in the skin, as shown in Figure 32, whether the flies were infected or not. Scientists are investigating this reaction in more detail to evaluate whether hypersensitivity to tsetse saliva affects the transmission of trypanosomes.

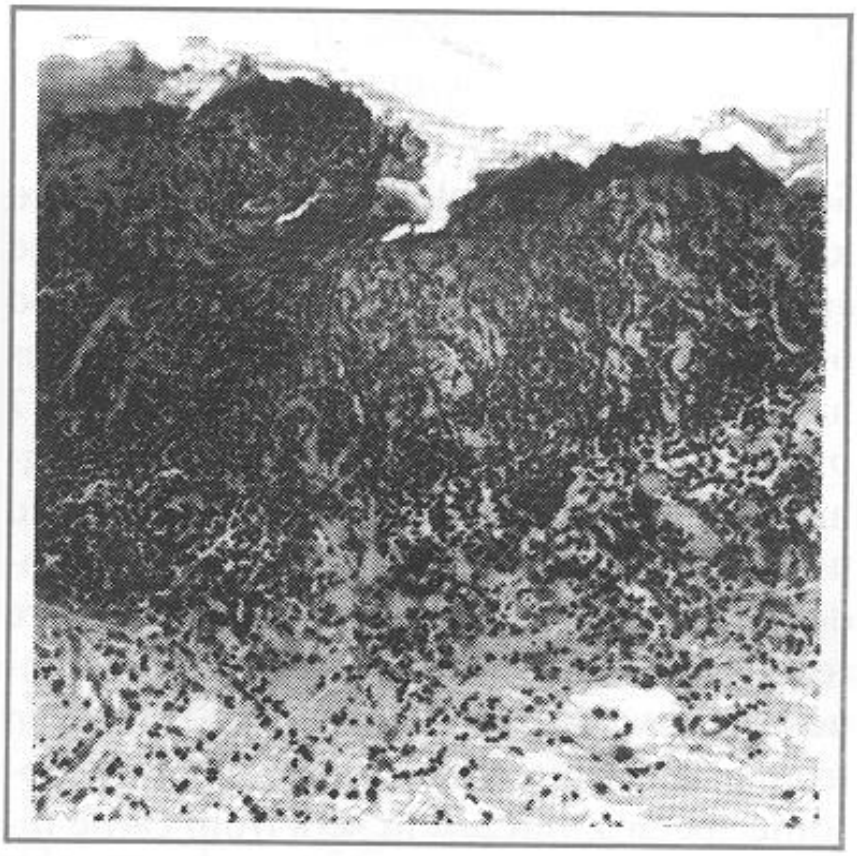

Figure 32. *The skin of a goat showing a severe hypersensitivity reaction 48 h after being bitten by an uninfected tsetse fly. This animal had been bitten on two previous occasions. There is*

extensive superficial necrosis of the epidermis and marked cellular infiltration of the dermis accompanied by severe oedema.

A special problem in trypanosomiasis epidemiology relates to the distinction between *T b brucei* which infects several species of domestic livestock, and *T b rhodesiense,* which infects humans. These two trypanosome subspecies are morphologically indistinguishable. Experiments have been carried out at ILRAD to identify *T b brucei* and *T b rhodesiense* infections by analysing parasite DNA with restriction enzymes. This work has been successful, but DNA analysis requires long and complex purification procedures which limits its usefulness under field conditions or for large-scale studies. For this reason, a simplified laboratory technique is being developed which can be used to identify different trypanosome species from samples of blood, tsetse saliva or isolated cells, as illustrated in Figure 33. Work is now in progress to develop a similar test which will distinguish the subspecies *T b brucei* and *T b rhodesiense,* and also *T b gambiense,* the other trypanosome subspecies which infects humans.

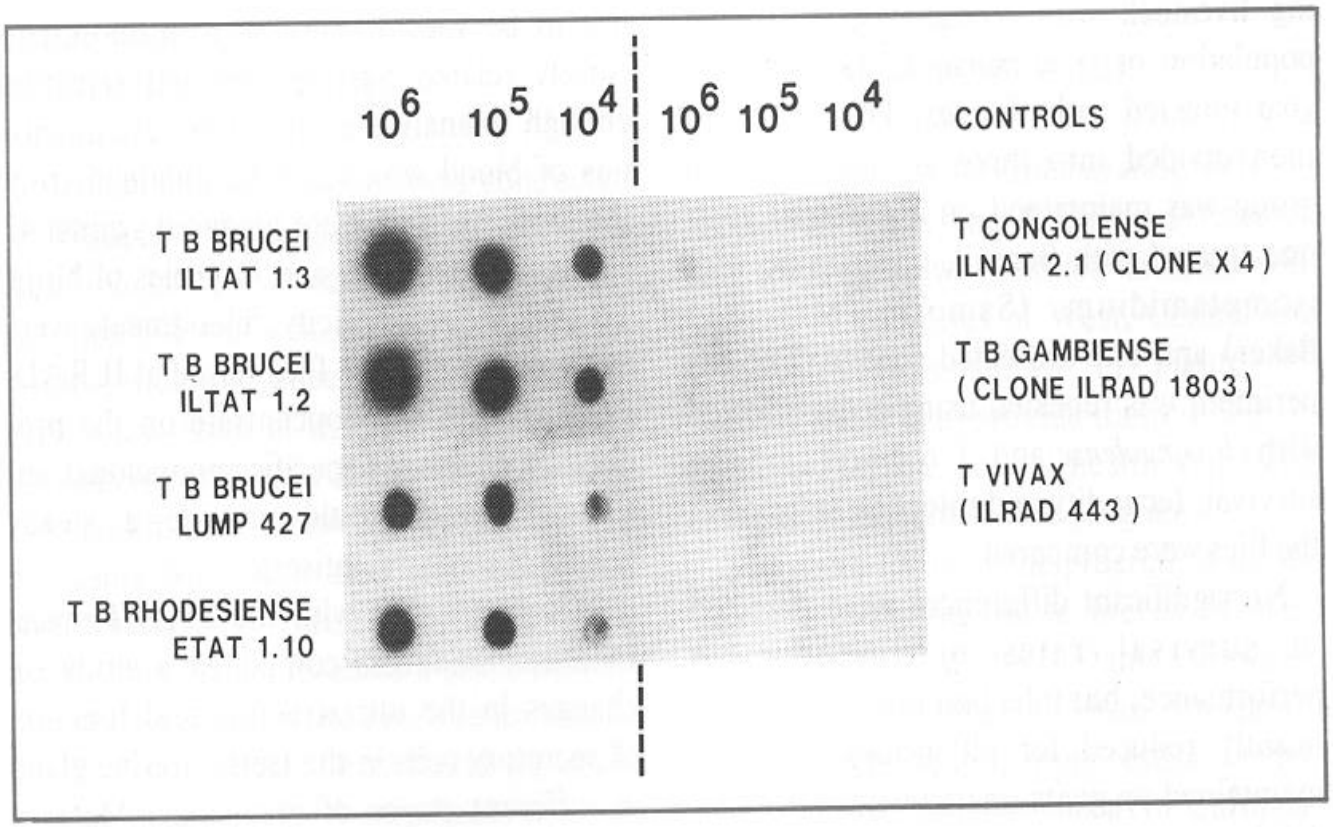

Figure 33. *Simplified illustration of a molecular hybridization technique for parasite identification. Suspensions of whole trypanosomes of four different stocks*—T b brucei *ILTat 1.3,* T b brucei *ILTat 1.2,* T b brucei *LUMP 427 and* T b rhodesiense *ETat 1.10—were diluted to different levels and applied to nitrocellulose filters. The filters were processed by the Southern blot method and the trypanosomes were hybridized with a probe for* T b brucei *ILTat 1.3 DNA. The figure shows that hybridization occurs only with parasites related to the specific probe. The degree of hybridization increases (darker spot) as the number of parasites increases. The method*

should be useful to distinguish the presence or absence of trypanosomes of a specific genotype, provided there is a minimum of 104 parasites in the sample.

The infectivity of different parasites for different host species may be linked to survival in the host bloodstream. Successive generations of *T b rhodesiense* populations have been observed which vary in terms of their sensitivity or resistance to the cytotoxic effects of human serum in vitro. This could indicate a variation in their potential viability in the human bloodstream. The reversibility of this sensitivity suggests that it is mediated by some protein or proteins which are synthesized in response to changing genetic information. ILRAD scientists have searched for differences in protein configurations between serum-sensitive and insensitive *T b rhodesiense* preparations using two-dimensional gel electrophoresis. However, no consistent differences have been detected yet.

Scientists have also observed a reversible pattern of sensitivity to human serum in *T b brucei* parasites isolated from laboratory animals and livestock. A cow was infected with a stock of *T b brucei* (TREU 667) which had previously been sensitive to the cytotoxic effects of human serum. Parasites appeared in the animal's bloodstream and cerebrospinal fluid. In vitro tests showed that parasites from the bloodstream were sensitive to human serum, while parasites from the cerebrospinal fluid were not. This suggests that *T b brucei* parasites which resist cytotoxicity in human serum may have an affinity for the central nervous system. Goats were infected with another derivative of this stock and parasites isolated from the bloodstream, the cerebrospinal fluid and the brain tissue all resisted the cytotoxic effects of human serum. Material has been collected which will be used to investigate whether changes in parasite sensitivity might be related to the expression of different surface antigens.

Studies on tsetse flies as trypanosome vectors

Tsetse studies at ILRAD concentrate on exploring improved methods for trypanosomiasis control. For instance, the release of sterile male tsetse flies has aroused considerable interest as a potential method of tsetse eradication. The success of this approach is based on the fact that female tsetse normally mate only once in their lifetime, so if most or all of the female flies in an area mate with sterile males the population will be eradicated. However, the possible impact on trypanosomiasis epidemiology of releasing large numbers of sterile males has not been fully explored.

Investigations in 1982 focused on the infection rates and transmission characteristics of male tsetse flies sterilized by gamma irradiation. Sterile and fertile males of three species-*Glossina morsitans centralis, G austen*i and *G tachinoides*—were fed on a goat infected with *T b brucei* and their survival rates, mating performance and transmission characteristics were compared. Ninety percent or more of the flies exposed to gamma irradiation were found to be sterile, but their mating performance was similar to that of fertile males. Differences in trypanosome infection rates between sterile and fertile males were marginal, and there were no differences in transmission characteristics between the two groups. These findings indicate that the release of sterile males as part of a tsetse control program could increase the immediate risk of trypanosomiasis. Similar studies are now in progress to examine the transmission of *T congolense and T vivax* by sterile male tsetse.

Another study concentrated on the possible impact on tsetse infection rates of treating livestock with trypanocidal drugs. A population of *G m morsitans* was fed on a goat infected with *T vivax*.. The flies were then divided into three groups and each group was maintained on a different goat, one treated with Berenil, one treated with isometamidium (Samorin-May and Baker) and one untreated control. The experiment was repeated using goats infected with *T congolense* and *T b brucei*, and the survival, fecundity and infection rates of all the flies were compared.

No significant differences were detected in survival rates or reproductive performance, but infection rates were significantly reduced for all groups of tsetse maintained on goats treated with trypanocidal drugs, as shown in Figure 34. This suggests that a carefully managed drug treatment program could significantly reduce the cyclical transmission of trypanosomiasis in situations where tsetse are largely feeding on livestock.

Original infection	Number infected	Maintained on goats treated with:	Number survived	Infection rates in surviving tsetse (%)
T vivax	200	Berenil	142	2.1
T vivax	200	Samorin	82	0.0
T vivax	200	Control	131	97.7
T congolense	200	Berenil	79	19.0
T congolense	200	Samorin	50	14.0
T congolense	200	Control	28	46.4
T b brucei	200	Berenil	89	0.0
T b brucei	200	Samorin	102	0.0
T b brucei	200	control	109	22.0

Figure 34. *Comparison of* T vivax, T congolense *and* T b brucei *infection rates in male and female* G m morsitans *infected as tenerals and then maintained on goats treated with Berenil or Samorin or on untreated control animals.*

To support epidemiological research in the field, a serological system has been developed to identify the animal species on which tsetse flies have fed. Such a system has to be specific enough to differentiate closely related host species and sensitive enough to analyse even the minute quantities of blood which can be obtained from the flies. Antisera were prepared against 42 species of mammals and 4 species of birds. To test their specificity, bloodmeals were analysed from tsetse flies raised at ILRAD. Further work will concentrate on the production of species-specific monoclonal antibodies which could provide a steady source of standard antisera.

In collaboration with the Swiss Tropical Institute, scientists completed a study on changes in the ultrastructure and function of secretory cells in the tsetse uterine gland at different stages of pregnancy. Material taken from *G m morsitans* raised at ILRAD was examined in Basel by electron microscopy. This study demonstrated a temporal correlation between cellular dynamics

and physiological events during pregnancy. The goal is to identify possible approaches to the control of tsetse populations by interfering with the reproductive process.

Epidemiological survey at the Kenya coast

An epidemiological survey was conducted in 1982 at a large dairy ranch near the Kenya coast. This location was chosen for an intensive study because the trypanosomiasis situation appeared fairly simple, with a low level of disease risk and little movement of livestock or wild animals.

A preliminary tsetse survey was carried out using bait oxen, biconical tsetse traps and searches for tsetse pupae and pupal cases. This survey revealed a small population of *G austeni* on the ranch in an area of dense riverine bush. Of 28 tsetse captured, 26 were dissected and 5 were found to be infected with trypanosomes: 2 with *T vivax* and 3 with *T congolense.* Four tsetse bloodmeals were analysed using antisera prepared at ILRAD. These were found to originate from a sheep, a cow, a warthog and a suni *(Neotragus moschatus).*

Blood samples were taken from a total of 5909 cattle on the farm. Of these, 1890 (32%) were anaemic, with a PCV of 30% or less. Twenty-five animals were found with trypanosome infections: 22 with *T congolense,* 2 with *T b brucei* and 1 with *T vivax*. Nearly all the infected cattle were kept near the area where the tsetse flies had been captured.

Twenty tracer cattle were brought to the tsetse-infested area and kept in an open pen. Fourteen of these became infected with *T congolense* about 22 days after exposure and were treated with Berenil. About 60 days after treatment, 16 animals became infected, including 13 reinfections and 3 first infections, and these were treated again. One animal became infected 3 times.

Collaborative epidemiology network

ILRAD scientists are collaborating with colleagues at ILCA and ICIPE in a research and training network which includes projects in 11 countries of West, Central and East Africa, as shown in Figure 35. ILRAD's role is to provide training and supervision for the animal health and tsetse survey components of the program.

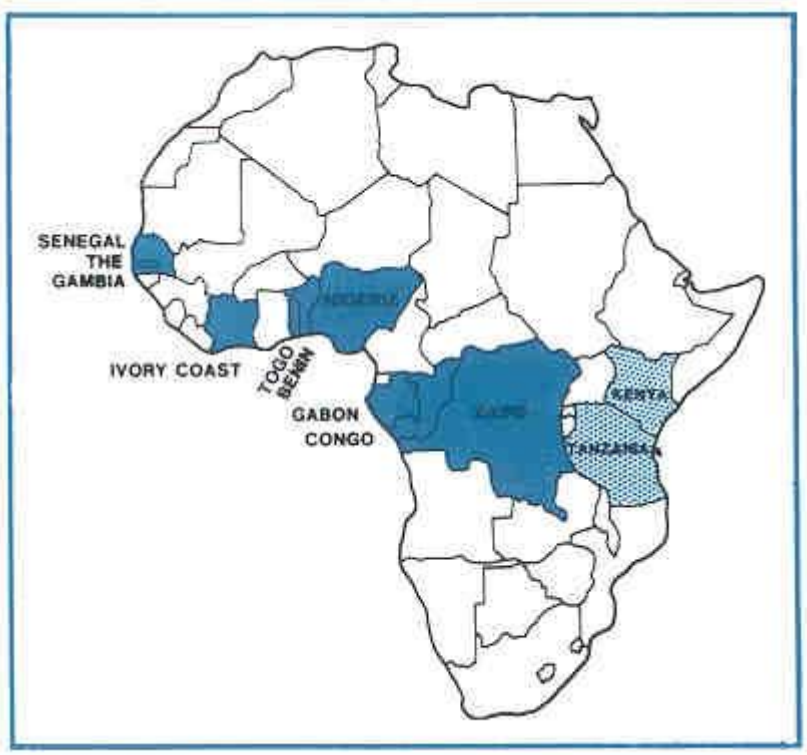

Figure 35. *Eleven countries in Africa where ILRAD is collaborating with ILCA and ICIPE in evaluating the impact of trypanosomiasis on livestock production. Major research sites are located in countries where trypanotolerant cattle are raised.*

The main focus of the program is on the indigenous taurine cattle breeds of West and Central Africa, the N'Dama (shown on the cover) and West African Shorthorn, which are significantly more resistant to trypanosomiasis than *Bos indicus* or European *Bos taurus* breeds. This trait, termed trypanotolerance, has been shown to be an innate characteristic. Comparative studies on these and other breeds are in progress in Ivory Coast, Nigeria, Zaire and Gabon and will be extended in 1983 to The Gambia, Senegal, Togo, Congo and Benin. Carefully selected production situations are being monitored where different breeds of cattle, sheep and goats are being raised under well defined management regimes and levels of trypanosomiasis risk. Similar studies are under way in Kenya and Tanzania focusing on indigenous breeds of cattle, sheep and goats.

Research Support

Tsetse Laboratory

Breeding colonies of 8 tsetse species were maintained at ILRAD in 1982, providing a good representation of the 30 species and subspecies found in tropical Africa. Colonies of G m centralis, G m morsitans, G austeni, G tachinoides, G palpalis palpalis, G fuscipes fuscipes, G brevipalpis and G pallidipes are maintained. All these species are being evaluated in terms of their capacity to transmit T vivax, T congolense and T b brucei to ruminants.

Tsetse flies raised at ILRAD are used for research on the role of tsetse as trypanosomiasis vectors and for collaborative research projects with other ILRAD scientists. In addition, 16,613 tsetse puparia and adult flies were supplied in 1982 to research centres in Kenya, Togo, Zambia, Austria and Switzerland.

The colony of G m centralis, originating from Singida in Tanzania, has been stabilized at a level of about 10,000 breeding females. This colony provides material for most trypanosomiasis research at ILRAD involving use of the vector. The G m morsitans colony, originating from the Kariba region of Zimbabwe, comprises about 1500 breeding females. The G austeni colony, originating from Zanzibar, and the G tachinoides colony, originating from Chad, are both maintained at a level of about 2000 breeding females.

A colony of G p palpalis was established in May 1982 from 1000 puparia received from IAEA in Vienna. The population originated in Nigeria. The performance of this colony has been good; the target breeding population of just over 2000 females was reached at the end of November.

Two colonies of *G f fuscipes* were established in 1982. One originated from wild flies and puparia collected in a forest on the shore of Lake Victoria in Uganda. This colony rose to 1300 breeding females by March 1982, declined rapidly and then began to increase again. The goal is to reach a breeding population of 1000 females in 1983. The other *G f fuscipes* colony was established in June 1982 with 500 puparia received from the Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux (IEMVT) in France. This stock originated from the Central African Republic. The number of breeding females had risen to 980 by the end of the year.

A colony of *G brevipalpis* was initiated in April 1982, originating from wild flies caught in the Kibwezi forest in Kenya. The breeding population increased gradually to 411 females by the end of November. A colony of *G pallidipes* was also established in April, originating from wild females caught in the Kibwezi forest. This colony fluctuated at around 250 females, but flies were then mated at an older age and breeding performance improved.

Tick Laboratory

ILRAD's tick laboratory maintains breeding colonies of two strains of *Rhipicephalus appendiculatus,* the vector for *T p parva* and *T p lawrencei,* and one strain of *Amblyomma variegatum,* the tick species which transmits *T mutans.* The tick laboratory routinely provides

ILRAD scientists with infected and uninfected ticks, tick salivary glands and other organs and tick-derived *Theileria* stabilates. In 1982, *Theileria* sporoblasts and sporozoites were harvested from over 40,000 infected ticks.

Several factors which affect tick growth, survival and infection rates are being assessed, including different tick strains, temperature conditions, different animal species and breeds used for tick feeding, the timing of feeding, the level of crowding and environmental factors influencing the host animal. The goal is to produce large numbers of ticks with as many parasites as possible. Staff from the tick laboratory also test the infectivity of tick-derived *Theileria* stabilates and monitor the course of experimental infections in cattle.

In addition to supplying tick and parasite material to scientists at ILRAD, in 1982 the tick laboratory supplied ticks to the Kenya National Veterinary Laboratories and the Veterinary Research Department of the Kenya Agricultural Research Institute.

Farm Animal Production

Scientists at ILRAD used about 400 cattle for research projects in 1982. About 85% of these were used for ECF research and 15% for work on trypanosomiasis.

ILRAD purchased a 13,000 ha ranch at the end of 1981 on the Kapiti plains about 80 km from Nairobi. Over 2000 Boran (*Bos indicus)* cattle were purchased with the ranch, including a breeding herd of 750 mature cows and 220 heifers. During the year, cattle handling facilities and water supplies were improved and housing was constructed for some of the ranch staff. To improve tick control on the ranch, a new spray race was installed and an existing one was renovated. By the end of the year, the breeding herd had grown to 786 mature cows and 595 heifers.

The ranch at Kapiti supplied ILRAD's research program with 335 calves and steers in 1982. Another 26 calves were bred at ILRAD, and 26 *Bos taurus* cattle were purchased from other ranches in Kenya for experiments which required these breeds. With the purchase of the ranch and expansion of cattle holding facilities at ILRAD, a steady supply of *Bos indicus* cattle was assured throughout the year. The supply of taurine cattle was interrupted for a period, however, when movement restrictions were imposed in Kenya due to an outbreak of foot-and-mouth disease.

Most research work involving cattle was carried out at Kabete, but 28 animals were taken to Kilifi as part of the ECF epidemiology project. Ten Zebu cattle were used for a collaborative trypanosomiasis research project, housed at the Kenya Veterinary Research Laboratories near ILRAD.

ILRAD scientists used about 400 small ruminants in 1982, including 370 goats and about 30 sheep. These animals are of local East African breeds, purchased from the area around ILRAD. In 1982, 95% were used for trypanosomiasis research. The screened accommodation available at ILRAD for trypanosomiasis experiments can accommodate 300 small ruminants. These facilities

were used to capacity in 1982. Expansion of the trypanosomiasis epidemiology program may require the construction of additional fly-proof housing.

Laboratory animal production

ILRAD's laboratory animal unit maintains breeding populations of mice, rats and rabbits, and small colonies of guinea pigs and gerbils. The mouse breeding program expanded from 5 inbred strains in 1981 to 12 strains in 1982. A total of 43,392 mice were produced in the course of the year, supplying all of ILRAD's research needs. Mice are used primarily to study the mechanisms of resistance to trypanosomiasis, as important genetic differences exist between inbred strains in terms of immune and other responses to the parasites. A random-bred strain of nude mice was imported in 1982, and a breeding program was initiated to develop inbred nude mouse strains. These are hairless animals with no thymus glands. Because their cell-mediated immune responses are inhibited, they are useful hosts for investigating the course of trypanosome infections and for comparative, studies with other mouse strains. However, because of their poor disease resistance, maintenance requirements are very high, and procedures in ILRAD's breeding unit have been modified accordingly.

One inbred rat strain is maintained, primarily to produce the trypanosomes required by ILRAD scientists. A strain of larger rats was introduced in 1982, replacing the strain used previously. During the year, a total of 25,075 rats were produced, sufficient for all of ILRAD's research needs.

Rabbits are bred primarily to support the tsetse and tick colonies. For this purpose, a new line of full-lop-eared rabbits has been introduced from the UK. A few rabbits are also used to produce sera for research and diagnostic purposes. Altogether, 450 rabbits were produced in 1982.

In addition to supplying nearly all the laboratory animals used at ILRAD, the breeding unit donated animals to a number of other research institutes. In 1982, these included ICIPE, the Kenya Trypanosomiasis Research Institute, the University of Nairobi, the National Museums of Kenya and an FAO project in Zambia.

Clinical and diagnostic Services

The diagnostic laboratory carries out several types of routine analysis for the research laboratories and animal production facilities. In 1982, 26,014 samples were received for analysis, more than twice the number received in 1981. These included 17,246 samples for serological, 5473 for bacteriological, 2284 for haematological and 1011 for helminthological analysis. This substantial increase in number of analyses performed in 1982 was due to the expanded ECF epidemiology project at the Kenya coast and an outbreak of salmonellosis which occurred in the cattle holding units at ILRAD.

Serological screening and titration were provided for antibodies to six bloodstream parasites found in cattle—*T p parva, T mutans, T b brucei, T congolense, T vivax and Anaplasma marginale.* Most routine screening for the *Theileria* and *Trypanosoma* species was by IFA test.

The ELISA test was also used to screen bovine sera for the three species of trypanosome. The card agglutination test was used to screen sera for A *marginale* antibodies.

During the year, 210 post-mortem examinations were carried out in the course of trypanosomiasis and ECF investigations. These included 111 cattle, 88 goats, 7 sheep and 4 animals of other species. Cases of salmonellosis, infectious bovine rhinotracheitis and muscular dystrophy were encountered in cattle, as well as the diseases under study. Urolithiasis and coccidiosis were found in goats.

In addition to services provided to ILRAD scientists, the diagnostic laboratory continued to assist other centres and projects in developing countries. *T p parva* schizont antigens, positive and negative control sera, and goat antibovine IgG and IgM conjugate were provided to the National Veterinary Laboratory in Uganda and to FAO projects in Burundi and Mozambique. Tests for *Theileria* and *Trypanosoma* antibodies were performed for a German (Federal Republic) technical aid project in Somalia.

Training and Information Services

ILRAD's training program is designed to support the development of scientific and field personnel who can extend research on trypanosomiasis, theileriosis and other pressing veterinary problems—primarily in Africa, but also in other parts of the world. Scientists and technicians are trained to carry out the most effective disease control programs possible with the means currently available. At the same time, they are prepared to initiate new field programs when improved control methods are ready for introduction.

Participants in the training program work closely with scientific staff within the scope of ILRAD's research mandate. Training opportunities are steadily increasing for scientists and senior technicians, post-graduate students and post-doctoral fellows, based on the availability of supervisory staff, laboratory space, equipment and accommodation.

In 1982, 14 scientists and technicians came to ILRAD for specialized training, for periods lasting from 1 week up to 1 year. Thirteen came from 9 African countries and 1 from India, and all were staff members of national universities, veterinary laboratories or research centres. Their training programs were planned on an individual basis according to the needs of their home institutes.

Three Kenyans from the Ministry of Livestock Development received training, two in basic haematological and parasitological techniques and one in the dissection of *Theileria*-infected ticks. One staff member from the Uganda Trypanosomiasis Research Organization was trained in histological techniques and another learned methods for maintaining laboratory animals. Two Zambians from the National Council for Scientific Research came to ILRAD to learn parasitological techniques relating to ECF. One Zairean from the Laboratoire de Parasitologie of the Université Nationale de Zaire was trained in parasitological and diagnostic techniques related to trypanosomiasis, and a Zimbabwean from the Veterinary Research Laboratory learned how to maintain *Theileria parasites* in culture. The head of the Parasitology Department at Addis Ababa University in Ethiopia received training in parasitological techniques related to trypanosomiasis, and a scientist from the Nigerian Institute for Trypanosomiasis Research learned techniques for maintaining *T vivax* in culture. A staff member from the Laboratoire Central Vétérinaire in Mali was trained in immunolgical and parasitological techniques, and a member of the Immunology Department of the University of Yaoundé in Cameroon received training on antigenic variation in trypanosomes, supported by the World Health Organization (WHO). From India, a professor of parasitology at Punjab Agricultural University received training in basic cell culture and specific techniques for cultivating *Theileria* parasites in vitro.

Fifteen post-graduate students worked with ILRAD scientists in 1982 on projects closely linked with ILRAD's research program. Post-graduate students are registered in a higher degree program, most often at the University of Nairobi. They work at ILRAD from 1 to 3 years and submit their projects as part of their degree requirements. In 1982, 10 students in this category came from Kenya, 2 from Uganda, and 1 each from Rwanda, Australia and Belgium.

Post-doctoral fellows are selected internationally, though emphasis is placed on recruiting well-qualified young scientists from African countries. They normally spend 2 years at ILRAD, and their work is expected to contribute directly to the research program. In 1982, 11 post-doctoral

fellows worked at ILRAD, including 2 from Germany (Federal Republic), 2 from the UK and 1 each from Kenya, Uganda, Zaire, India, Belgium, Japan and the USA.

Altogether in 1982, 40 students, scientists and technicians received individual training at ILRAD, 8 more than in 1981. Thirty of these trainees were from African countries, including 5 from francophone Africa.

In addition to training opportunities offered on an individual basis, a 6-week training course was held at ILRAD in October and November on the 'Use of Immunological Methods in the Diagnosis of Protozoan Diseases'. Fourteen participants attended this course from 10 African countries, including 5 from Kenya and 1 each from Liberia, Mali, Nigeria, Senegal, Sudan, Tanzania, The Gambia, Uganda and Zambia. The participants reviewed basic biochemical and immunological concepts with ILRAD scientists and learned how to produce antibodies, isolate and purify immunoglobulins, assess specificities, prepare antigens and cultivate different parasite species. They were also taught to perform a range of serodiagnostic tests, with emphasis on the diagnosis of protozoan parasites.

ILRAD scientists helped conduct a 10-week training course in 1982 in collaboration with colleagues from ILCA and ICIPE. This was the first of a series of training sessions for field staff taking part in the ILCA/ILRAD livestock productivity and trypanotolerance research network. The course covered the extraction, analysis and interpretation of data on animal production in areas under trypanosomiasis risk. Four scientists participated in this session, one from Ethiopia and three from Zaire, and one participant from Nigeria received the same training on an individual basis earlier in the year. A training manual, 'Livestock Productivity and Trypanotolerance', was prepared in English and French in connection with the course.

ILRAD's training program provided support for three scientists to attend a 1-week Workshop on 'Trypanotolerance and Animal Production', held in Avetonou, Togo in May 1982. The workshop was sponsored by the Centre de Recherche et d'Elevage, the Government of Togo and the German (Federal Republic) Agency for Technical Cooperation and Foundation for International Development. One of the participants sponsored by ILRAD came from Senegal, one from The Gambia and one from Uganda.

The ILRAD library maintains a specialized collection of scientific literature, comprising about 2400 books, subscriptions to 198 periodicals, and a selection of maps and materials on microfiche. The library is used by ILRAD scientists and trainees and by researchers working at other institutions in the Nairobi area, including ICIPE, the University of Nairobi, the Kenya National Veterinary Laboratories, the Kenya Trypanosomiasis Research Institute, and the Veterinary Research Department of the Kenya Agricultural Research Institute.

The international periodicals are probably the most important component of the library collection. Journals are received by air every week, covering topics in such fields as parasitology, immunology, biochemistry, entomology, cell biology and veterinary medicine. Many of these are not available in any other library in Kenya. Local scientists have access to these journals in the ILRAD library or through an active inter-library loan system. Through this system, ILRAD scientists also have access to other libraries in the area, some of which are

associated with older research institutions and thus have collections going back for many years. If staff members require articles which are not available locally, these are purchased from the British Library Lending Division.

In 1982, the scientific staff published 35 articles in international journals, 1 chapter in a book and 2 papers in conference proceedings. The 1981 annual report was prepared in English—and for the first time in French—and a series of brochures was issued in both languages describing ILRAD's research and training activities and listing all scientific publications. In addition, ILRAD's communications staff prepared press releases and articles for outside publication and arranged for coverage of ILRAD's research activities on Kenya television. Articles and conference papers published by ILRAD scientists are kept in the library, and copies distributed on request. Exchanges of publications with other specialized libraries have expanded steadily since ILRAD's library was established in 1976.

The regular seminar series continued in 1982, with sessions held every 2 weeks. These were well attended by scientists from ILRAD, the University of Nairobi and other national and international research centres in the area. ILRAD staff members also participated in numerous international conferences and gave lectures on trypanosomiasis and theileriosis research to participants in training courses sponsored by other organizations.

ILRAD's research and training activities were presented to a large audience in September 1982 in an exhibit prepared for the Nairobi International Show. This show is sponsored every year by the Agricultural Society of Kenya. Thousands of people visited ILRAD's exhibit from all over Kenya.

ILRAD staff members have played a key role in setting up the Nairobi Cluster, an informal organization of University departments, government laboratories and research institutes working on trypanosomiasis and ECF in Kenya. Eleven institutions in the Nairobi area are members. The Cluster issued a newsletter in 1982 and sponsored a scientific meeting on 'Strategies and Recent Developments for the Control of Trypanosomiasis and East Coast Fever', held at ILRAD. The meeting was opened by the Minister for Livestock Development, the Hon Mr. Paul Ngei. Participants included scientists, government officials, livestock producers, veterinarians and representatives of drug companies.

Research projects were carried out in collaboration with agencies and organizations in many parts of the world. In Kenya, ILRAD scientists were involved in research and technical collaboration with the Ministry of Livestock Development, ICIPE, the Veterinary Research Laboratories, the Veterinary Research Department of the Kenya Agricultural Research Institute, the Kenya Trypanosomiasis Research Institute, the OAU's Interafrican Bureau for Animal Resources, ILCA, the University of Nairobi's Faculties of Medicine and Veterinary Medicine and the Clinical Research Centre of the Kenya Medical Research Institute. Research was also conducted in collaboration with the Centre de Recherche et d'Elevage in Togo, the Centre de Recherches sur les Trypanosomiases Animales in Upper Volta and the national veterinary laboratories of Burundi, Malawi, Mozambique, Rwanda, Tanzania, Zambia and Zimbabwe. Outside Africa, ILRAD scientists collaborated with colleagues at the Prince Leopold Institute for Tropical Medicine, the Vrije Universiteit Brussel and the Libre Universite de Bruxelles in

Belgium, the Swiss Tropical Institute, Cambridge, Glasgow and Edinburgh Universities in the UK and the Universities of California, Iowa and Washington State in the USA. Several collaborative projects are described in the text of this report.

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N.Saigar

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V.Shitakha

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R.Thatthi

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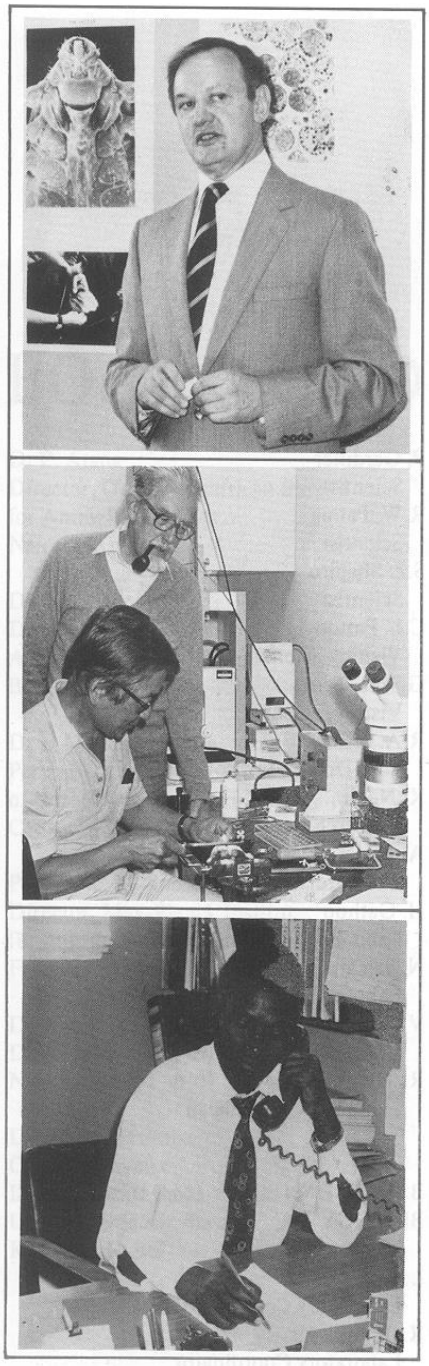

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R.O. Williams

Laboratory Coordinator

O.Moi Yoi

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Scientist

G.Matthyssens

Visiting Scientist

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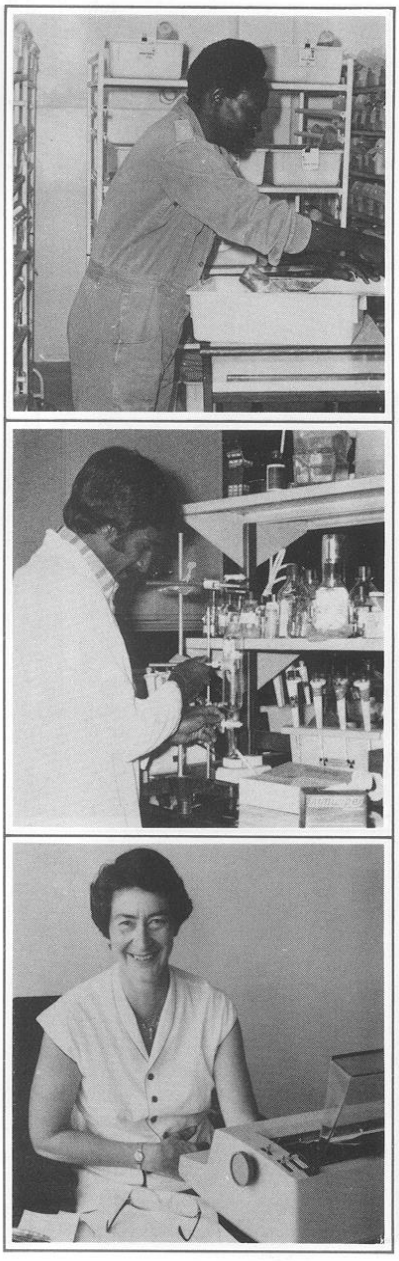

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J.G.R.Ocama

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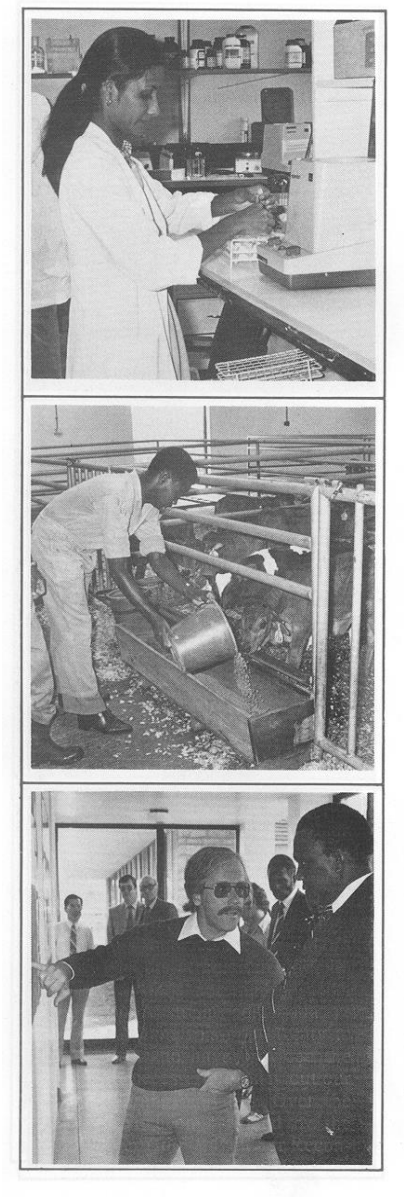

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W. Vogit

Laboratory Coordinator

B. Otim

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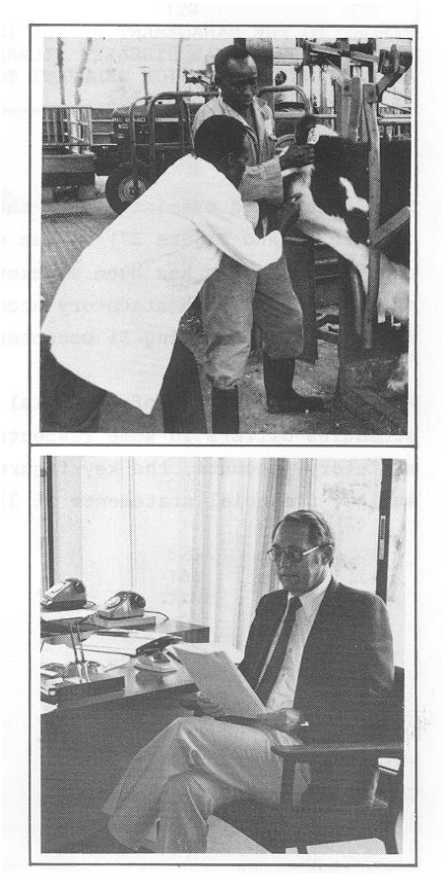

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Report to the Management of the International Laboratory for Research on Animal Diseases (ILRAD) On Internally Prepared Accounting Information Relevant to the Financial Years Ended 31 December 1981 and 1982

From a detailed examination of the accompanying schedules (Figure 36 and Figure 37) we can confirm that the information contained therein has been extracted from the books and records of ILRAD from which statutory accounts were prepared for the financial years ending 31 December 1981 and 1982.

Whereas the analysis of financial data in the accompanying schedules differs in some respects from that disclosed in the statutory accounts, the key figures remain consistent with the audited financial statements at 31 December 1981 and 1982.

Price Waterhouse 25 March 1983

Certified Public Accountants

Figure 36. Summary of costs by program and activity (US$ '000).

	1982	1981
Research		
Parasitology-Trypanosomiasis	446	469
Biochemistry	483	554
Cell Biology	382	344

Immunobiology	285	455
Parasitology-Theileriosis	449	493
Pathology	448	483
Tsetse Laboratoy	301	312
Tick Laboratory	93	76
Electron Microscopy	129	117
Collaborative Research	142	64
Total Research	3158	3367
Research Support		
a. Farm Animal Production	573	458
Laboratory Animal Production	131	28
Clinical and Diagnostic Service	63	84
Radio Isotope and Central Core Services	400	303
Total Research Support	1167	973
Training and Conferences	526	301
Library and Information Services	267	166
Administration		
b. Board of Directors	70	81
Office of the Director General	319	416
Finance	379	509
Personnel	73	54
Purchasing	257	223
Total Administration	1098	1283
General Operations		
Engineering	523	536
Transport	185	206
Services	275	313
Food and Housing	33	233
Stores	54	67
Total General Operations	1070	1355
Contingency	166	-
Total Core Costs	7452	7445
Special Project - Netherlands	141	123

Figure 37. *Summary of sources and application of funds (US$ '000).*

	1982	1981
Core Operating Funds		
Unrestricted		
World Bank (IBDR)	280	1910
Germany (Federal Republic)	717	770
United Kingdom	559	540
Canadian International Development Agency (CIDA)	545	505
Switzerland	391	412
Norway	325	380
Belgium	185	258
Rockfeller Foundation	-	100
Netherlands	295	230
Australia	286	265
Sweden	200	185
Ireland	-	100
International Atomic Energy Agency (IAEA)	5	-
Balance From Previous Year	2648	2131
Earned Income Applied in Year	101	184
Total Unrestricted Operating Funds	6534	7970
Restricted		
United States Agency for International Development (USAID)	2400	3100
United Nations Development Fund (UNDP)	799	694
Belgium	99	77
Total Restricted Operating Funds	3298	3871
Total Unrestricted/Restricted Operating Funds	9835	11841
Transfer to Capital/Funds	(1550)	(1749)
Net Unrestricted/Restricted Operating Funds	8285	10092
Capital Funds	1550	1749
d. Transferred from Core Operating Funds	45	637
Unexpended Balance from Previous Year	709	709
Total Capital Funds	2304	3095
Special Project-Netherlands	60	52
Total Funds	10649	13239